





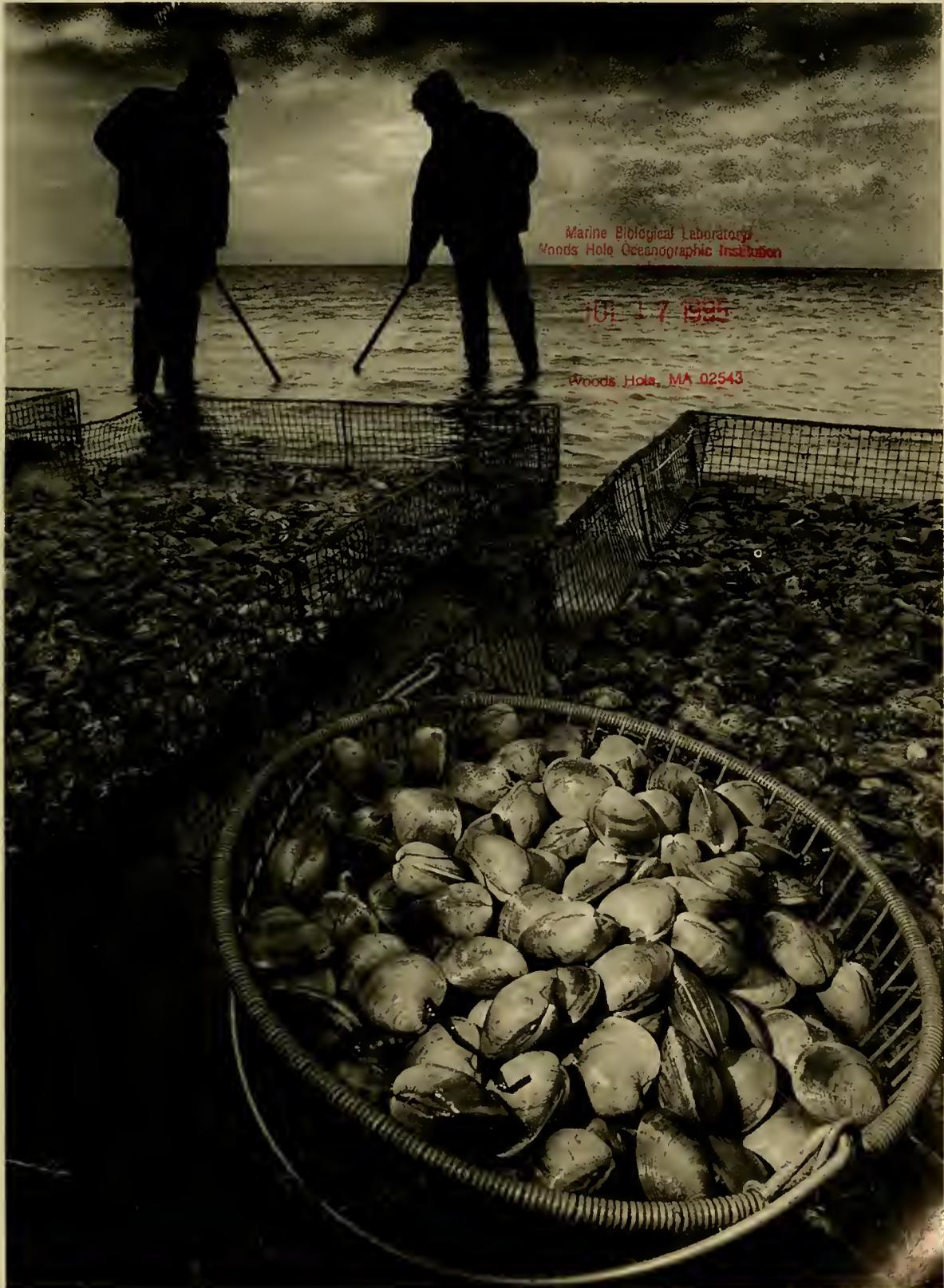




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**IN MEMORIAM**  
**Paul Peter Yevich**  
**1924-1994**

The familiar cigar and beret and that ever strong and bellowing voice will no longer grace the laboratory or scientific meetings. Paul Yevich, an international authority on comparative histopathology and diseases of marine bivalve molluscs, died December 3, 1994, in Wakefield, RI, following a heart attack. He was 70 years old.

Paul grew up in Berwick, PA, the youngest of 10 children of the late John M. and Theresa (Pavlova) Yevich. During these formative years, he honed his sharp wit and keen argumentative skills in endless supertime philosophical debates ("round 'n round") with his siblings. He developed his medical interests in high school by hitchhiking 25 miles to Geisinger Hospital in Danville. There he was befriended by the doctors he questioned, and they allowed him to use the medical library. He wrote a paper detailing all of the then-known theories of cancer which was so thoroughly researched that a teacher had it published. (Unfortunately, it has been lost.)

At Pennsylvania State College (now University) he continued his research under the tutelage of Dr. George B. Newman. His primary interest at this stage was hematopoiesis, particularly as it occurred in rabbits. His academic career was interrupted by World War II. He served in the Army in the European Theater; first in the infantry, then as an interpreter of German and Russian after the war ended. After being honorably discharged from the Army, he returned to Penn State and completed his B.A. degree in 1949.

His career in comparative histopathology began at the Army Chemical Center in Edgewood, MD (1950-1960), where he worked with Dr. James R. M. ("Hamish") Innes, Chief of Pathology, on the toxicology of warfare chemicals in diverse animals. He conducted histopathologic studies on animals exposed via inhalation, oral, cutaneous, subcutaneous, and peritoneal methods to toxicants such as nerve gases, riot agents, rocket fuel, oxygen, carbon monoxide, mustard gases, nitrosamine, psychochemical agents, smokes, radiation, and various industrial pollutants. He left the Army Chemical Center to attend graduate school at Michigan State University to study diseases of blood and blood-forming organs, but after six months decided to return to the government with the Public Health Service in Cincinnati, OH.

As Research Histopathologist in the Occupational Health Research and Training Facility, Paul conducted histopathologic studies on various species of animals in relation to disposition and retention of particulates in the lung, as well as studies on black lung disease in humans. He lectured on the physiology of particulates in human lung and industrial lung diseases during training courses offered by the Public Health Service, and served as a consultant to other agencies on problems of industrial lung diseases.

In 1966, Clarence Tarzwell asked Paul to join him at the newly established National Marine Water Quality Laboratory (NMWQL) in West Kingston, RI, as Research Biologist (Histopathology) for the laboratory's toxicology program, part of the Federal Water Quality Administration (now the U.S. Environmental Protection Agency). When the laboratory became EPA's Environmental Research Laboratory (ERLN) and moved to its present location in Narragansett in 1973, Paul continued to develop the histopathology unit, creating a state-of-the-art laboratory, testing new procedures for fixing and processing marine animals, and training numerous technicians and students in histotechnique and comparative histopathology. The Histopathology Unit was completely equipped to study the morphological and functional anatomy of marine fauna and flora using methods in histology, histochemistry, electron microscopy, and autoradiography. In an average year, the unit processed over 500 animals for routine histopathological examination, producing 15,000 to 20,000 slides.

Paul experimented to adapt mammalian techniques or to find new methods for preparing marine animals. Some of his more notable

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## THE REPRODUCTIVE BIOLOGY OF PIPI, *PAPHIES AUSTRALIS* (GMELIN, 1790) (BIVALVIA: MESODESMATIDAE). I. TEMPORAL PATTERNS OF THE REPRODUCTIVE CYCLE

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**ABSTRACT** The reproductive cycle of the New Zealand pipi (*Paphies australis*) was investigated over two years (May 1991 to April 1993) in a small harbour, in northeastern New Zealand. Samples of pipi gonads were processed using standard histological techniques, and the resultant sections placed into five developmental categories (early active, late active, mature, partially spawned and indeterminate). Gametogenesis was similar in both years, beginning in autumn with most pipi in early active stages of development. By late winter many pipi were mature. Spawning commenced in early spring and continued through spring and summer. From a sample of 104 pipi collected in spring 1992 for investigation into the length at sexual maturity, all pipi were found to be sexually mature above 40 mm shell length, but some individuals between 30–40 mm also had gonads with sex cells present. This sample had a sex ratio significantly biased towards females. Further analysis revealed that juveniles (below 40 mm shell length) had a significantly higher proportion of females than males, but adults (above 40 mm shell length) had a sex ratio of approximately 1:1. No evidence of hermaphroditism was observed.

**KEY WORDS:** *Paphies*, pipi, reproductive cycle, sexual maturity, clam, temporal

### INTRODUCTION

Knowledge of the reproductive cycle of any marine bivalve species is essential to an understanding of the duration and timing of spawning activity. This in turn may potentially influence the distribution and abundance patterns of juvenile and adult populations, and provides a basis for growth estimates that are useful in the management of the species (Shaw 1965, Baron 1992). Successful culture of clams is also dependent on a good understanding of the reproductive cycle of natural populations (Eversole 1989).

Clams are an important resource internationally (Manzi and Castagna 1989, Grizel 1993). In New Zealand, clams form a small but growing commercial fishery (Cranfield and Michael 1992, 1993). Most species remain largely unexploited and information on their basic biology remaining relatively scarce and only available from unpublished reports (e.g., Cranfield and Michael 1992, 1993, Cranfield et al. 1993, Cranfield et al. 1994).

The clam family Mesodesmatidae is represented in New Zealand by four species: *Paphies australis* (pipi), *Paphies ventricosa* (toheroa), *Paphies subtriangulata* (tuatua) and *Paphies donacina* (deep water tuatua). They are perhaps the most familiar infaunal bivalves of beaches throughout New Zealand, in the Chatham Islands and in the Auckland Islands (Powell 1979). They are abundant both intertidally and subtidally often reaching densities well in excess of 1000 m<sup>-2</sup> (Venus 1984, Grant 1994, Hooker and Creese 1995a) and resurgent populations dominate sandy shores around New Zealand. They form a large part of the recreational shellfish harvest and support a small commercial fishery (Redfearn 1974, 1975, Haddon 1989).

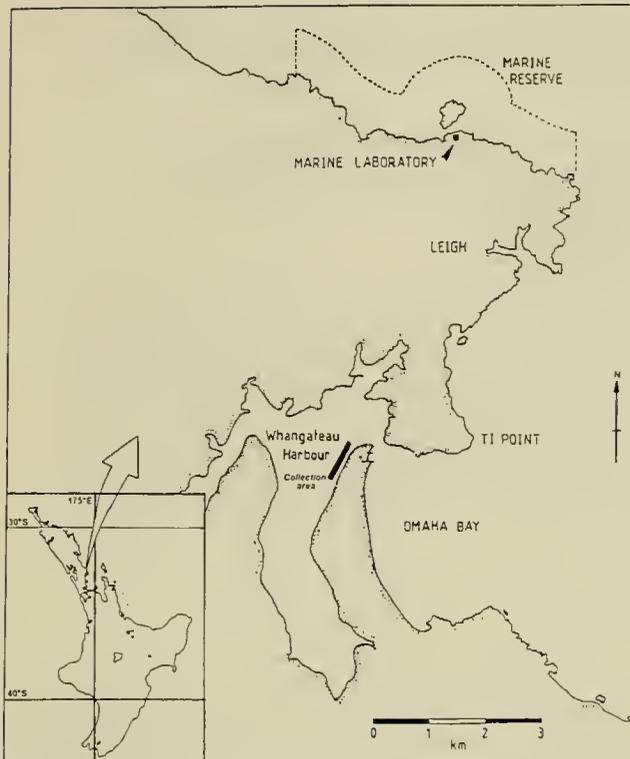
Despite the importance of *Paphies* spp, very little scientific information is available on even their basic biology. There is, however, a small amount of information on their reproductive biology, although for pipi this is not in the primary literature. Populations of toheroa (*P. ventricosa*) have been shown to contain mature individuals throughout the year, although there was a tendency for an increased proportion of the population to be mature in late spring with spawning occurring shortly after and continuing through the summer (Redfearn 1974). Condition indices for to-

heroa have generally shown an increase in condition over autumn and winter, reaching a peak in late winter to early spring, and then dropping (corresponding to spawning) to a minimum in late summer (Rapson 1952, Cassie 1955, Redfearn 1974). Rapson (1952) found that all toheroa above 3 inches (76 mm shell length) were mature and mature eggs were found in individuals of 0.5 inches (13 mm). Redfearn (1974) confirming these findings, also found that 20% of three months old toheroa (15 mm) were mature, 75% were mature at nine months (32 mm) and all were mature by 15 months (47 mm).

Grant (1994) found two spawnings, during one year, for tuatua (*P. subtriangulata*): a minor one in March 1993 and a major one in October/November 1993. The major spawning peak roughly corresponded to a preliminary investigation of spawning of tuatua by Greenway (1981). Grant (1994) suggested that the spring spawning may have been induced by small scale changes in water temperature.

Although there is no formal information on the size of the recreational harvest of pipi, circumstantial evidence suggests it far exceeds the commercial catch. As part of a series of unpublished reports, the reproductive cycle of pipi (*P. australis*) has been studied for an environmental bio-monitoring program (Dickie 1986a). Pipi were sampled from four sites (three intertidal and one subtidal) in the Whangarei Harbour (55 km north of the Whangateau Harbour) in northeastern New Zealand. The study concluded that there was a very distinctive spawning period in late spring to early summer, and a subsequent spawning later in the summer. This was followed by a short resting stage and gametogenesis started again in the late autumn. Booth (1983) reports, in an appendix, a condition index for pipi using the ratio of the wet weight of tissue to the wet weight of tissue plus shell through time. His results showed high condition from February to May and a decline in condition during May to June, possibly indicating spawning.

The study of reproductive cycles in marine invertebrates forms the basis of most ecological studies on species biology. Reproductive studies are essential to an understanding of life history characteristics and demographic studies as well as providing essential information for fisheries management and aquaculture ventures. In



**Figure 1.** Map of North Island of New Zealand showing the location of the Whangateau Harbour, the area where all pipi samples were collected and the University of Auckland's Leigh Marine Laboratory.

this study the reproductive cycle of pipi was investigated over a two-year period from May 1991 to April 1993 in the Whangateau Harbour on the northeastern coast of New Zealand (Fig. 1). The reproductive cycles of clam populations are known to vary with latitude; therefore, reproductive information on pipi from more northerly localities may not be applicable to pipi populations in the Whangateau Harbour.

The aims of this study were to describe the reproductive cycle and the spawning season of pipi in the Whangateau Harbour. This was done in order to help us to establish links with larval abundances, any observed recruitment patterns, to provide information for larval culture experiments and to establish a spawning time for age at length information for demographic studies.

#### MATERIALS AND METHODS

Specimens of pipi were collected from the Whangateau Harbour (Fig. 1) over two years from May 1991 to April 1993. Monthly samples of adult pipi were collected subtidally by SCUBA in 7–10 m water depth, with additional sampling over suspected spawning periods in spring and late summer. In the laboratory, shell lengths were recorded and the shell broken with pliers to extract the live animals, which were then fixed in Bouin's solution for histological preparation.

Due to the difficulty of separating the gonad tissue from the surrounding somatic tissues in clams, gonadal somatic indices (which rely on relative weights of gonad to other tissues) are not as common as in other molluscs (Eversole 1989). A more common method in many bivalves (and clams in particular) is to divide the reproductive cycle into a number of stages based on microscopic examination of histological sections (Sastry 1979, Eversole 1989).

To allow comparisons with other clam species, histological techniques were chosen for our study of the reproductive cycle of pipi.

#### Histology

The gonad of the pipi is located along the posterior margin of the foot and extends anteriorly towards the digestive diverticula (Fig. 2). The gonad extends into the foot and when mature, enlarges well into the foot. Dickie (1986a) has previously tested whether histological sections from throughout the gonads of pipi were representative of the reproductive state of the whole gonad. He selected two animals, one female and one male, and sectioned them eight times at regular intervals transversely through the gonad. Five replicate counts of various follicular tissues were made on each section. This showed that most of the variation present was attributable to differences within a section rather than between sections. He concluded that it was not critical where the section was taken within the gonad and that there was little to be gained by processing more than two replicate sections within an individual.

#### This Study

Initially, from May 1991 to October 1991, samples of ten females were processed, and from November 1991 to April 1992, samples of thirty pipi (males and females) were processed. Two tissue sections were taken at a standard point halfway between the tip of the foot and the digestive diverticula (Fig. 2). All tissue sections were blocked in paraffin and sectioned at 5–7  $\mu\text{m}$  thickness and stained with Mallory Heidenhain solution. The two sections from the same individual were mounted on a single slide. All histological slides were examined under a light microscope at 40 $\times$ , 100 $\times$  and 400 $\times$  magnification. Each slide (individual) was put into one of six categories, modified from Loosanoff (1942), Shaw (1962), Ropes and Stickney (1965) and Ropes (1968). Sometimes, more than one stage occurred within an individual. When this occurred, the stage was determined from the condition of the majority of the section. Due to the initial difficulty of accurately staging spawned individuals, a sample of pipi was collected during November 1993 when spawning had been observed in the wild. These were taken back to the laboratory where they were artificially induced to spawn. After spawning, the pipi were preserved and sectioned as above.

#### Length at Sexual Maturity

Preliminary data collected during 1991 suggested that pipi were reproductively mature in spring. Therefore an additional sample of pipi was collected, in spring 1992, from the Whangateau Harbour and brought back to the laboratory. Pipi were collected over the full range of available sizes and placed into 5 mm size classes. They were then opened and fixed in Bouin's solution. All pipi were sectioned as described above and the sections examined for the presence of any sex cells and evidence of hermaphroditism (both male and female gametes co-occurring within the same individual). Animals were defined as mature if sex cells were present.

#### RESULTS

##### Description of Phases

Four stages could be clearly defined for male and female pipi. A fifth stage was also present (indeterminate) in which animals could not always be reliably sexed.

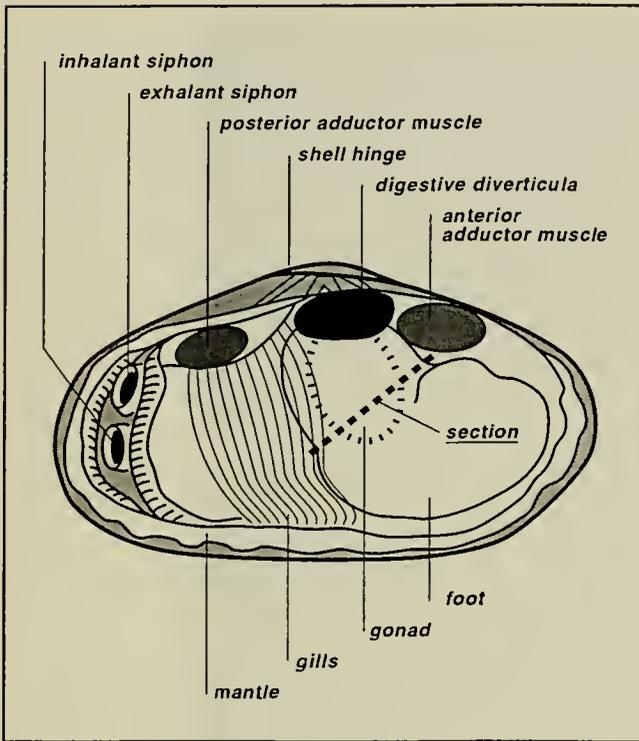


Figure 2. Anatomy of pipi with the left valve removed showing the area of the gonad where the histological section was taken.

#### Female Gonad

1. Early active phase (Fig. 3A,B). There is a lot of ovogenic activity. Many small primary oocytes are attached to thickened follicle walls, often to the follicles by a stalk or broad cytoplasmic base. The nucleus occupies most of the cell volume. Follicle cells are sometimes present and fill the lumen. There are always mature oocytes and ova present. The gonad volume is small.
2. Late active phase (Fig. 3C,D). There are many more mature oocytes and ova than in the early active phase. Follicle walls are thin. The oocytes are attached to the follicle walls by a thin stalk. There are still many primary oocytes. Follicle cells have disappeared.
3. Ripe/mature phase (Fig. 3E,F). Numerous mature ova are in the lumen. Follicle walls are thin and there is little ovogenic activity. Gonad volume is large, occupying much of the space within the body wall and extending well into the foot.
4. Spawning/partially spawned phase (Fig. 3G,H). Follicle walls are thin and sometimes ruptured. Free ova and oocytes are still frequent except in the spent individuals. The walls of some follicles have not contracted, so there are large spaces in the lumen and few ova or oocytes. As gametogenesis is a cyclic process so this phase can be present with stage three or overlap with the first active phase.

#### Male Gonads

1. Early active phase (Fig. 4A,B). There is a thin layer of spermatogonia against the thin follicle walls. Proliferation of spermatocytes and spermatids; the centre of the lumen often with small numbers of spermatozoa. Total volume of gonad is small.

2. Late active phase (Fig. 4C,D). Follicles expanded with spermatogonia lining the follicle walls. Dense area of spermatocytes and spermatids occupying approximately one third to one half of the lumina. The centre of most lumina filled with dense radiating bands of spermatozoa.
3. Ripe/mature phase (Fig. 4E,F). A thin layer of spermatogonia line the follicle walls; in some there is none. Spermatocyte and spermatid layers are less thick than in the previous stage. Dense bands of spermatozoa fill the majority of the lumina and often swirl into the centre.
4. Spawning/partially spawned phase (Fig. 4G,H). Spermatogonia as in stage three. Spermatozoa still occupy the lumina but have numerous gaps. Spawning stage has few if any spermatocytes or spermatids.

#### Indeterminate Gonads

5. Indeterminate phase (Fig. 5A,B). Few or no sex cells present. In most cases the sex cannot be determined.

#### Reproductive Cycle

The mean shell length of pipi sampled was 62.5 mm ( $\pm 0.12$  se). The reproductive cycle for female pipi in the Whangateau Harbour was similar in both years sampled (Fig. 6a). Gametogenesis started in autumn with the majority of individuals in the early developmental stage. At the end of autumn the gonads of most females were in the late developmental stage. By early winter (June) a few of the pipi had become mature. The proportion of mature females increased through winter and by late winter/early spring the population had spawned. Spawning continued through to late summer, at which time the largest proportion of indeterminate individuals was present in the population.

The 1991 year had two major spawnings, one in September/October and the other in November/December. Spawning was characterized by a change from a high proportion of mature individuals (stage 3) to a high proportion of spawned (stage 4) and early active individuals (stage 1). During this year the proportion of mature females increased to 90% by early September 1991; the rest were in the late active stage (stage 2) (Fig. 6a). This was followed by the first major spawning event, leaving no mature individuals in the population. Spawning (shown by the decrease in the proportion of mature individuals and the increase in the proportion of spawned individuals) continued while the proportion of mature individuals increased and the female population reached another peak of maturity, with 70% mature on the 7 November 1991. Over the next day the population spawned (Fig. 6a). The gonads quickly recycled to the early active stage, and by late November over 80% of the population were in late active stage.

Development of the female gonads during the 1992 year followed a similar pattern to that noted in 1991. By June the gonads had matured again and by July 1992 most (70%) were mature and the rest were in the late active stage (Fig. 6a). Spawning came earlier than in 1991, with some spawned clams appearing in August 1992 when less than 10% of the population was mature. The gonads quickly recycled and by September over 50% of the population was mature. Again the proportion of mature female pipi increased rapidly to a maximum of over 80% in November 1992. Further spawning activity occurred between November and December 1992. Minor spawning continued until early autumn of 1993 when most of the population were in the early active stage of development.

Male pipi followed a similar reproductive cycle to females

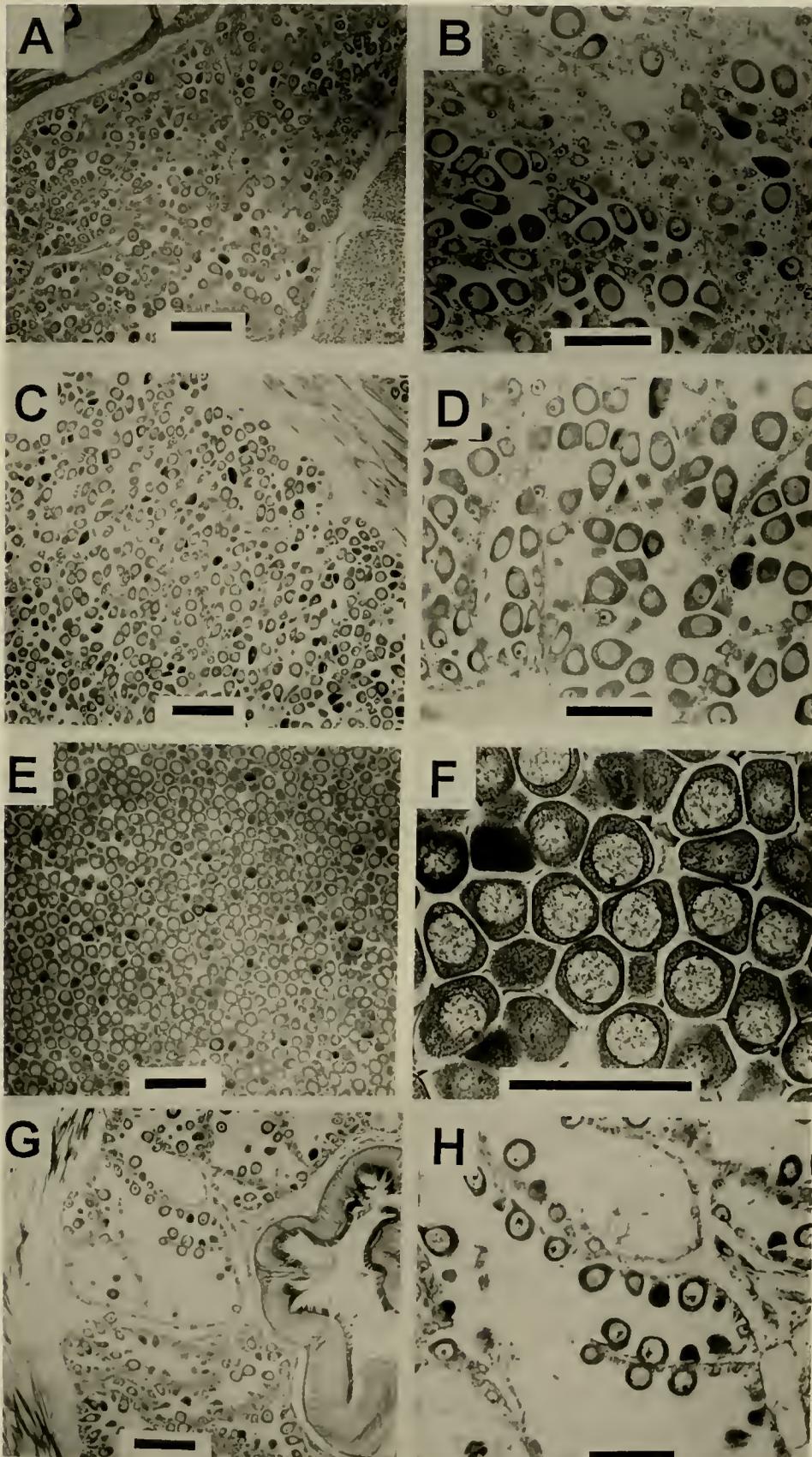


Figure 3. Photomicrographs of female pipi gonad showing: (A,B) the early active phase, (C,D) the late active phase, (E,F) the mature phase, (G,H) the partially spawned phase. Scale bars: (A,C,E,G) 200 µm, (B,D,F,H) 100 µm.

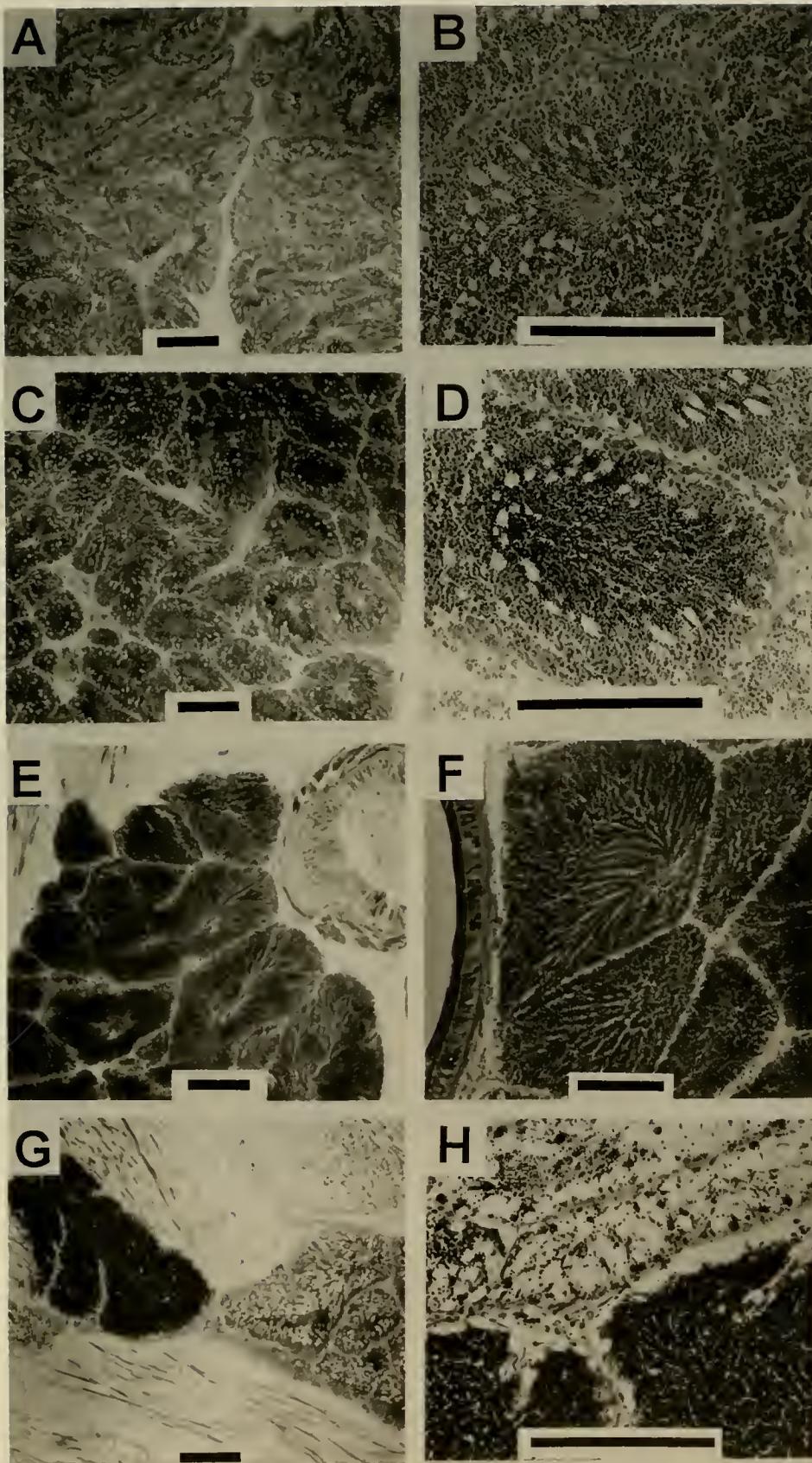


Figure 4. Photomicrographs of male pipi gonad showing: (A,B) the early active phase, (C,D) the late active phase, (E,F) showing the mature phase, (G,H) the partially spawned phase. Scale bars: (A,C,E,G) 200  $\mu\text{m}$ , (B,D,F,H) 100  $\mu\text{m}$ .

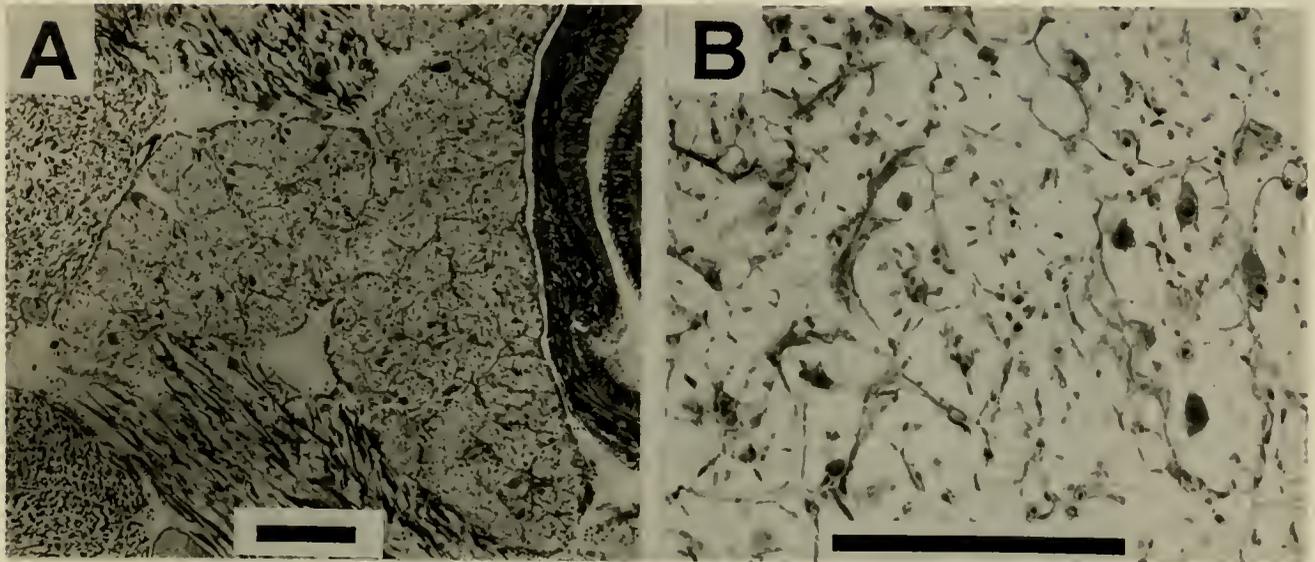


Figure 5. Photomicrographs of pipi gonad showing the indeterminate phase. Scale bars equal: (A) 200  $\mu\text{m}$ , (B) 100  $\mu\text{m}$ .

(Fig. 6b). Partially spawned male individuals were more easily identified than those of females. This accounts for the larger proportion of spawned males found compared to females over the period September 1992 to February 1993 (Fig. 6b). Fully mature males were present only from June 1992 to February 1993, clearly delineating the period of reproductive activity.

A long resting stage was not apparent in either sex. Indeterminate gonads were more common during autumn of each year, at the end of the spawning season, possibly indicating a short resting stage in a few individuals. However, there was a very low proportion of the indeterminate stage, and generally there were mature oocytes and spermatozoa present throughout the year (Fig. 6a,b).

There seemed to be little correlation between spawning activity and average seawater temperature taken at the near-by Marine Laboratory. Laboratory experiments (Hooker 1995) have shown that sexually mature pipi will spawn when given a thermal shock and a dilute sperm solution. Pipi spawned in the laboratory in November 1993 gave a clear indication of the spawning stage. At

no time were pipi found that had completely spawned in the wild or in the laboratory, all having retained the majority of their mature oocytes and spermatozoa after spawning.

Small scale fluctuations in water temperature were recorded at site 2 during October (spring) 1993 and showed that the temperature could fluctuate by 3.5°C over a daily cycle (Grant 1994). During warm spring days the harbour temperature could be up to 5°C warmer than the incoming oceanic water (pers. obs.). The demarcation was often very sharp with warm harbor water being separated by the incoming cooler oceanic water by only 2–3 m. This would also happen in reverse on the ebb tide although there was not as sharp a boundary between the warm and cool water.

#### Length at Sexual Maturity

Mature pipi (from 30 mm to 51 mm shell length) were found in all size classes sampled (Table 1). However, of the 9 mature pipi found in the smallest length class (37.5%), 8 of them had virtually no sex cells present. It was only by very intensive searching at high magnification (400 $\times$ ) that the sex cells were identified at all. Therefore it was concluded that this length class constitutes the very early state of maturity for pipi. Virtually all (96%) pipi above 40 mm were classed as mature. For all pipi sampled greater than 50 mm in length (from the reproductive cycle) the percentage mature was always at least 96%. It was concluded that the population would never reach 100% maturity due to the odd indeterminate individual found in the adult population. It was not possible to differentiate between these and immature individuals. The overall sex ratio of the pipi in the sample collected from site 3 in early November 1992 had significantly more females than males (Table 2). Closer examination found that pipi below 40 mm shell length had significantly more females than males while those above 40 mm were not significantly different from a 1:1 sex ratio. There was no evidence of hermaphroditism in any pipi sampled.

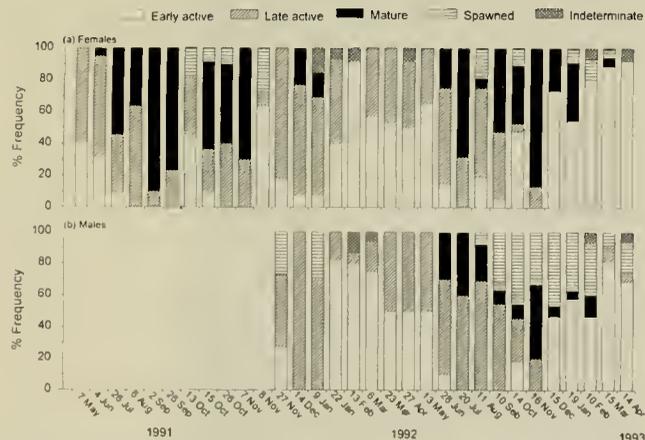


Figure 6. Proportional bar chart showing the reproductive cycle of pipi in the Whangateau Harbour. (a) females; May 1991 to April 1993, (b) males; November 1991 to April 1993. Indeterminate individuals that were not able to be sexed were omitted from the analysis.

#### DISCUSSION

The reproductive cycle of clams has been extensively studied using a variety of techniques. Gonadal somatic indices have not been commonly used in clams (e.g., Eversole et al. 1984), due to

the difficulty of separating the gonad from the surrounding tissue. Macroscopic examination of gonads (e.g., Caddy 1967, Gilbert 1978, McGreer 1983) needs to be backed up by histological examinations. Although this can be time consuming and expensive, it confirms that the observations are a result of gametogenic processes. Various forms of condition indices have been used to describe the reproductive cycles of clams (Ansell et al. 1980, Booth 1983, Broom 1983). This is acceptable if the condition index is used in conjunction with histological analysis, but otherwise it may give spurious results as condition indices are not designed to give reproductive information. The most common method of determining the reproductive cycle in clams is by the use of histological sections. Some authors have used quantitative measures of various gonad tissues (e.g., Keck et al. 1975, Brousseau 1978, Eversole et al. 1980, Breese and Robinson 1981, Robinson and Breese 1982), but the majority of authors have used qualitative developmental stages to describe the reproductive cycle of clams (e.g., Shaw 1962, 1965, Porter 1974, Feder et al. 1979, Jones 1981, Manzi et al. 1985, Robinson 1992, Kanti et al. 1993). One of the main difficulties with this latter technique is that it assumes that a continuous process can be adequately represented by subdivision into discrete categories. Despite the difficulty in assigning the stages to some individuals, the phases were a realistic way of describing the gametogenic process in pipi. Previous accounts of reproductive cycles in clams have successfully used three to six categories (Corni et al. 1985, Brousseau 1987, Sephton 1987, Heffernan et al. 1989, Hesselman et al. 1989, Rowell et al. 1990). There is no universal protocol in the number of categories chosen as it will depend on the species being investigated.

The mature phase was the most easily identified of the developmental stages and was easily distinguished from other stages. The most accurate representation of the spawning season can be gained by following the relative drop in the proportion of mature individuals through time. All spawned individuals encountered in this study were only ever partially spawned, often spawning only a very small percentage of the available ova or spermatozoa. Sex cells left over after spawning may be extruded later, undergo cytolysis, be maintained until the next spawning (Eversole 1989) or any combination of these depending on the species of clam. The presence of a low percentage of indeterminate adult individuals throughout our study may be due to a number of reasons. Firstly, they may have been individuals that had completely spawned, with little or no sex cells left in the gonads. This is unlikely as it would be expected that some evidence of spawning (e.g., such as large spaces in the lumen where the follicle walls had not contracted) would remain in at least a few individuals, furthermore the artificially spawned animals always retained large numbers of mature

TABLE 1.  
Length at sexual maturity.

Size Range	Total Number	Number Mature (%)	% of Total
30–35 mm	24	9 (37.5)	11.1
36–40 mm	27	21 (77.8)	25.9
41–45 mm	25	24 (96.0)	29.6
46–50 mm	28	27 (96.5)	33.3
Total	104	81 (77.8)	100

Numbers and percentages of mature pipi in 5 mm size classes.

TABLE 2.

Sex ratios of juvenile (<40 mm shell length) and adult (>40 mm shell length) pipi from length at sexual maturity data.

Size Range	Total	Male	Female	Indeterminate	Chi Squared
Juveniles (31–40 mm)	51	7	23	21	8.53*
Adults (41–50 mm)	53	19	32	2	3.31
Total (31–50 mm)	104	26	55	23	10.38*

$\chi^2$  critical value (1 df) at  $P = 0.05$  is 3.841.

\* Indicates significance at 95%.

eggs. Secondly, they may have been immature individuals that had never matured. Thirdly, they were possibly inactive individuals that had undergone resorption of residual sex cells. This is also unlikely as no characteristic signs of resorption were noticed at any time. Also, it does not explain why the majority of normally spawning individuals retain the sex cells until the next spawning. Finally, a resting or inactive stage before the onset of gametogenesis may be present. We were not able to distinguish between these possibilities with the present data.

Pipi in the Whangateau Harbour do not have a discrete spawning period, rather a prolonged breeding period from late winter to late summer with spawning activity predominantly occurring in spring. This is similar to reports for other Mesodesmatidae in New Zealand (Redfearn 1974, Dickie 1986a, Grant 1994). Booth (1983), however, suggested a very different pattern; he found pipi from northeastern New Zealand were mature (had a high condition index) from February to May and declined during May and June possibly indicating spawning. This discrepancy to other studies may be due to the more northerly location than other studies, an artifact of the condition index he used, or simply that his condition index does not accurately describe the reproductive cycle of pipi. Spawning in pipi can be a major synchronized event, as shown by the rapid change that occurred over the period November 7 and 8 1991, and highlights the advantage of the non-rigid sampling strategy that allowed additional sampling at crucial times. We believe that Mesodesmid clams from New Zealand do not have well-defined reproductive cycles. Spawning in pipi is not a discrete event but a series of partial spawnings of the population, over weeks or possibly months probably dependent on local environmental influences. This mode of reproduction makes precise prediction of spawning events, or of subsequent recruitment pulses very difficult.

Temperature is one of the main environmental factors thought to control gametogenesis and spawning in bivalves (Galtsoff 1964, Porter 1964, Keck et al. 1975, Eversole et al. 1984, Eversole 1989). During the two years over which this study took place, water temperatures were unseasonably low with temperatures reaching the lowest recorded level in twenty five years at the adjacent Leigh Marine Laboratory (Heath 1993). This needs to be taken into account when considering the reproductive activity of pipi over longer time scales.

During the spring of 1993, when the water temperature within the Whangateau Harbour was known to fluctuate the most, a small sub-population of pipi in shallow water (2–3 m) at the entrance of

the harbour were seen spawning. Pipi had dug themselves out of the sand and were elevated a few centimeters above the substratum. The pipi would then stretch out their siphons and emit a short burst (seconds) of gametes. An individual would repeat this action a number of times over an hour or more. Spawning individuals could be sexed by the appearance of the gametes as the eggs were visible to the naked eye and the sperm appearing as a milky cloud. Tuatua (*Paphies subtriangulata*) were also spawning within a few centimeters of the pipi. This spawning activity was observed to continue over the following weeks. Grant (1994) was able to closely link the observed spawning of tuatua to the small scale fluctuations in water temperature produced from the effect of the warm spring sunny days and the relatively cool water temperature. Toheroa and tuatua are also known to spawn with sharp changes in water temperature (Redfearn 1982, 1987). Small scale fluctuations in water temperature might also be a major cue to the spawning of pipi (Hooker and Creese 1995b).

Sexual maturity in this study was assessed as a function of shell length. Although no assessment of age at length was made in the present study, sexual maturity may be a function of age, length or some combination of age and length. It is likely, however, that length can be used as a convenient indicator of sexual maturity (Eversole 1989). Sexual maturity is also likely to be a function of maximum size with larger species becoming mature at an increased size. Pipi from the Whangateau Harbour rarely grow above 70 mm shell length (Hooker and Creese 1995c). Pipi from Whangarei Harbour tend to be larger as individuals over 75 mm are common (Dickie 1986b, 1987, Haddon 1989). Eversole (1989)

suggests that sexual maturity is generally reached in clams at approximately 25% of the maximum size. Pipi in the Whangateau Harbour reached maturity between 30–40 mm which is well above one quarter (closer to half) of maximum shell length for pipi at this locality. Further discussions of this relationship will have to await studies of sexual maturity in pipi and other *Paphies* species from other localities. In this study, more female pipi developed at a smaller size than male pipi. Possible reasons for this are either that female pipi simply develop before male pipi, or pipi may be protandric hermaphrodites, starting as females with a proportion changing to males later, resulting in a 1:1 adult sex ratio. However this is unlikely as no evidence of hermaphroditism was seen despite extensive searches of the histological sections. Other studies on clams have revealed the opposite pattern, with male clams developing before females (Caddy 1967, Porter 1974, Eversole et al. 1980, Ropes et al. 1984, Rowell et al. 1990, Menzel 1991). A possible reason for this difference is that females require more time (i.e., a larger size) to meet the greater trophic cost of becoming female (Russell-Hunter and McMahon 1975).

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## THE REPRODUCTIVE BIOLOGY OF PIPI, *PAPHIES AUSTRALIS* (GMELIN, 1790) (BIVALVIA: MESODESMATIDAE). II. SPATIAL PATTERNS OF THE REPRODUCTIVE CYCLE

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**ABSTRACT** A population of adult pipi, *Paphies australis*, was sampled monthly from February 1992 to January 1993 at three subtidal sites within a small harbour in northeastern New Zealand. Analysis of the reproductive cycle used standard histological techniques and all specimens were assigned to one of six categories: early active, late active, mature, partially spawned, empty, and parasitized. The reproductive cycle was similar between sites, with an extended spawning period of late winter to summer. It was concluded that the reproductive cycle of pipi in the Whangateau Harbour was synchronized at all three sites sampled. A visual maturity index gave the same overall pattern of reproductive cycle as the histological analysis, but was found to be inaccurate mainly due to a poor recognition of the mature stages. It was concluded that the visual index was of limited value in accurately assessing the maturity of an individual. The sex ratio of adult pipi was not significantly different from 1:1. A small percentage (0.87%) of pipi were infected by a digenetic trematode fluke that, in most cases, totally castrated the individual.

**KEY WORDS:** Reproductive cycle, *Paphies*, pipi, visual index, spatial, clam

### INTRODUCTION

Most of the studies cited in a review of bivalve reproductive cycles (Sastry 1979) base their conclusions on data collected from only one location. Many are also based on samples collected over a single annual cycle. Commercial species of oysters, mussels, scallops and clams are an exception, with numerous studies being done over several years in many localities by many researchers (see reviews by Andrews 1979, Sastry 1979, Dohmen 1983, Eversole 1989). Previously we described the reproductive cycle of the New Zealand pipi, *Paphies australis*, over two annual cycles (Hooker and Creese 1995a). In this paper the variability in this pattern is examined at three sites within one harbour, separated by hundreds of meters. Although pipi have a continuous distribution linking these three sites, the sites vary topographically from the narrow mouth of the harbour, which is exposed to stronger currents, (Hooker and Creese 1995b), higher densities and more wave action (Hooker and Creese 1995c), to a sheltered site further up the harbour near the end of their distribution in the harbour (where there are likely to be slight differences in temperature, salinity and water movement). Because these environmental conditions (such as salinity, food availability and water temperature) are known to affect various aspects of the reproductive cycles of bivalves, such as the initiation of gametogenesis and the onset of spawning (Loosanoff and Davis 1950, 1963, Giese 1959, Ropes 1968, Sastry 1968, Cain 1975, Chanley 1975, Nagabhushanam and Dhamne 1977, Sastry 1979, Chanley 1981, Kassner and Malouf 1982, Eversole 1989, Castagna and Manzi 1989, Heffernan et al. 1989, Newell 1991), it was considered possible that details of the reproductive cycle might vary across the distribution of the pipi population.

When comparing a number of sites, it is advantageous to be able to use a quick and simple index of reproductive activity. Visual indices, based on the outward appearance of the gonad, have been used in a number of studies of clam reproduction (Porter 1964, Caddy 1967, Gilbert 1978, McGreer 1983, Baron 1992). The spatial variation in the reproductive cycle of pipi in the Whangateau Harbour provided the opportunity to assess the usefulness of these types of visual indices, and allowed an in-depth view of

the reproductive cycle and spawning dynamics of pipi over their extent in the harbour.

The use of histological sections also allowed an assessment of the extent of parasitic trematode infection within a population of infaunal bivalves. Digenetic trematode parasites are well known and common in clam populations and are known from almost all marine bivalve species (Kinne 1983, Lauckner 1983). Bivalves are usually a primary or secondary intermediate host for the trematode parasite, but sometimes act as the definitive host (Lauckner 1983). Many commercially important clam species are known to act as host for trematode parasites (Gibbons and Blogoslawski 1989, Lauckner 1983). For example, the hard clam *Mercenaria mercenaria* is a second intermediate host for trematode parasites with the definitive host being shore birds (Cheng et al. 1966, Cheng 1967). This parasitism, however does not seem to adversely affect the populations of the clam (Menzel 1989, 1991).

The aims of this paper, therefore, are to assess the spatial pattern of the reproductive cycle within one year, evaluate the usefulness of visual indices in determining reproductive condition of pipi, establish the extent and distribution of trematode infection of pipi, and determine the sex ratio of pipi at three sites in the Whangateau Harbour (Hooker and Creese 1995a).

### MATERIALS AND METHODS

Specimens of pipi were haphazardly collected from the centre of the bed at monthly intervals from February 1992 to January 1993. Thirty pipi, of 55 mm shell length or greater (mean = 62.6 mm, se = 0.08), were collected subtidally, by SCUBA from three sites varying in water depth from 3 m to 7 m below low tide level (Fig. 1) in the main channel of the Whangateau Harbour. Animals were transported to the nearby Marine Laboratory where shell length was recorded and the shell broken with pliers to extract the live animal. A visual gonad index (see below) was estimated and the animal was preserved in Bouins solution for histological preparation.

#### Histology

All pipi were sectioned and examined as described in Hooker and Creese (1995a). Animals were then placed in one of six cat-

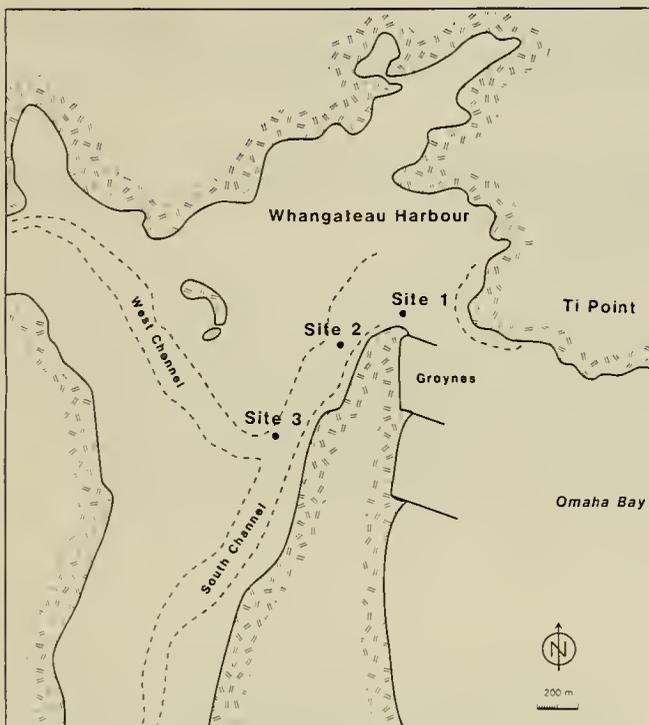


Figure 1. Map of the Whangateau Harbour showing the location of the three sites where monthly samples of adult pipi were collected for the reproductive study.

egories derived from the detailed temporal analysis presented in Hooker and Creese (1995a): early active, late active, mature, partially spawned, indeterminate and parasitised. All but the last category (parasitised) are described in Hooker and Creese (1995a). In the parasitised category the gonad had, in most cases, been invaded by a parasitic digenetic trematode and the sex could not be determined (Fig. 2).

#### Visual Index

Histological examination of gonadal material is often time consuming and expensive. In some circumstances it may be desirable

to quickly assess the gross reproductive condition of an individual. A visual index was developed to establish how easily and accurately an individual could be assessed for reproductive maturity. To help make the comparison between the visual index and the histological analysis more rigorous, those individuals that were placed in the categories "parasitised" and "partially spawned" were deleted from the analysis. The "parasitised" category did not constitute a developmental stage and the "partially spawned" individuals may appear as one of any three of the visual categories depending on how much it had spawned. "Indeterminate" individuals were equated to the "early active" stage as they would externally appear similar (i.e., very little gonad). A Spearman's rank correlation was conducted between the histological analysis and the visual index.

With one of the valves removed, each individual was briefly examined and put into one of three categories:

1. Immature. There was little gonad material present. The body was limp and the gut was often visible through the body wall.
2. Developing. Gonad tissue was visible through the body wall. The gonad was not totally full and extended. In most specimens the gut was not visible through the body wall.
3. Mature. The gonad was very extended and visible. The color of the gonad was pink in females and creamy white in males. The gut was not visible.

Each individual was fixed in Bouin's solution, and then preserved in 70% ethanol ready for histological preparation.

#### Sex Ratio

Pipi were sexed by microscopic examination of histological slides. A Chi square, goodness-of-fit-test was used to test the hypothesis of equal representation of males and females.

## RESULTS

#### Reproductive Cycle Between Sites

There was a clear reproductive cycle of pipi in the Whangateau Harbour (Fig. 3). The pattern showed a trend of increasing development of the gonad from February, with the population composed of predominantly early active or late active individuals, to

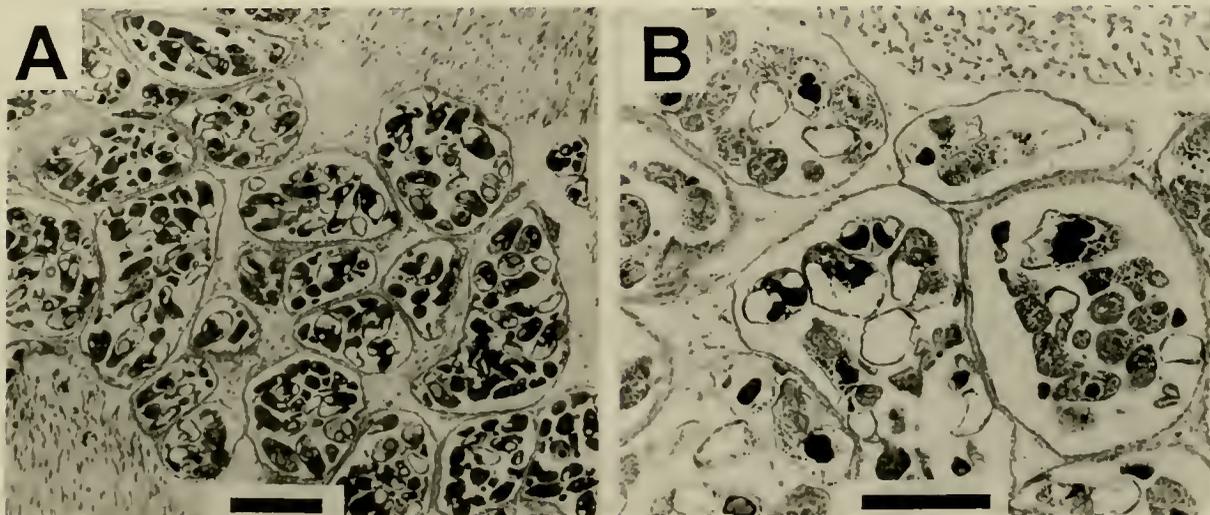


Figure 2. Photomicrograph of a pipi gonad showing the parasitic fluke. Scale bars equal: (A) 200  $\mu\text{m}$ , (B) 100  $\mu\text{m}$ .

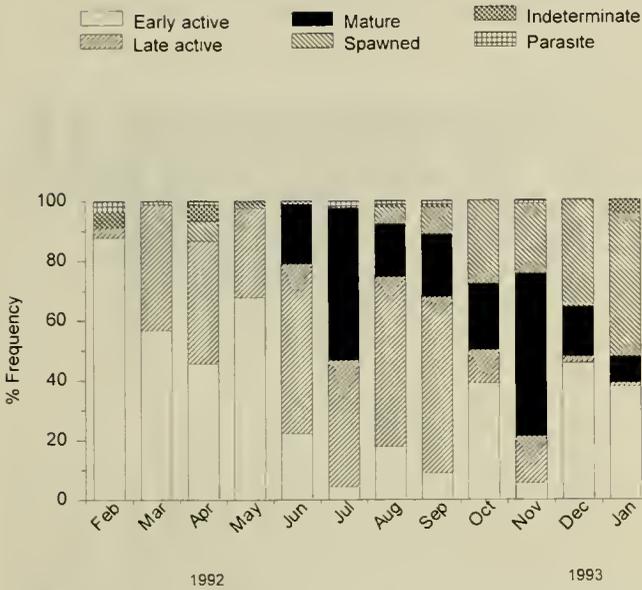


Figure 3. Proportional bar chart of the histological data showing the reproductive cycle at all sites and male and female pipi combined in the Whangateau Harbour.

June with the first fully mature individuals appearing in the population. From then on there were varying numbers of all stages at the three sites. Mature individuals occurred consistently at all sites from June to January. The number of mature individuals in the population peaked twice, during July and November. There were two main spawning periods (as shown by the appearance of partially spawned individuals and the loss in numbers of mature individuals) one in July/August and the other in November/December.

All three sites show the same general reproductive pattern as described above (Figs. 4 and 5). There were, however, slight variations in the relative numbers of each stage at each site. Early and late active stages dominate the population from February to May for both sexes at all sites. Females at site 1 did not become mature until July whereas the other two sites had mature females by June (Fig. 4). Also, site 1 had less mature females in November and also less in July and September compared to sites 2 and 3, but more spawned individuals over the spring and early summer. Male pipi followed the same basic reproductive pattern as females at each of the three sites (Fig. 5). Again, as with the females, the males at sites 2 and 3 were more similar than at site 1—no mature males were found in either September or January as they were in sites 2 and 3. There were less mature males than females. Indeterminate individuals were present at all sites (Figs. 4 and 5) with a total of 21 (1.8%) found overall. There was a slight trend towards increasing numbers of indeterminate individuals towards the entrance of the harbour (site 1).

**Visual Index**

The reproductive cycle shown by the visual index was similar to that shown by the histological analysis (Fig. 6), but was inaccurate. The first mature individuals visually identified were in June, at the same time as the histological analysis identified maturity. Although spawning individuals could not be identified, the drop in the number of mature individuals in December was probably due to spawning. As with the histological analysis all three

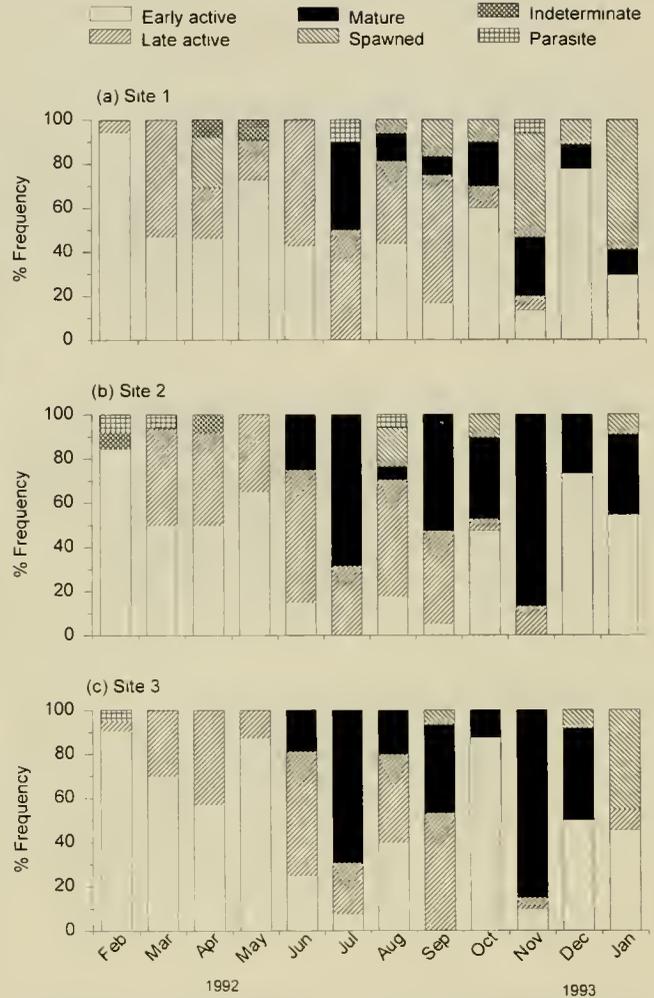
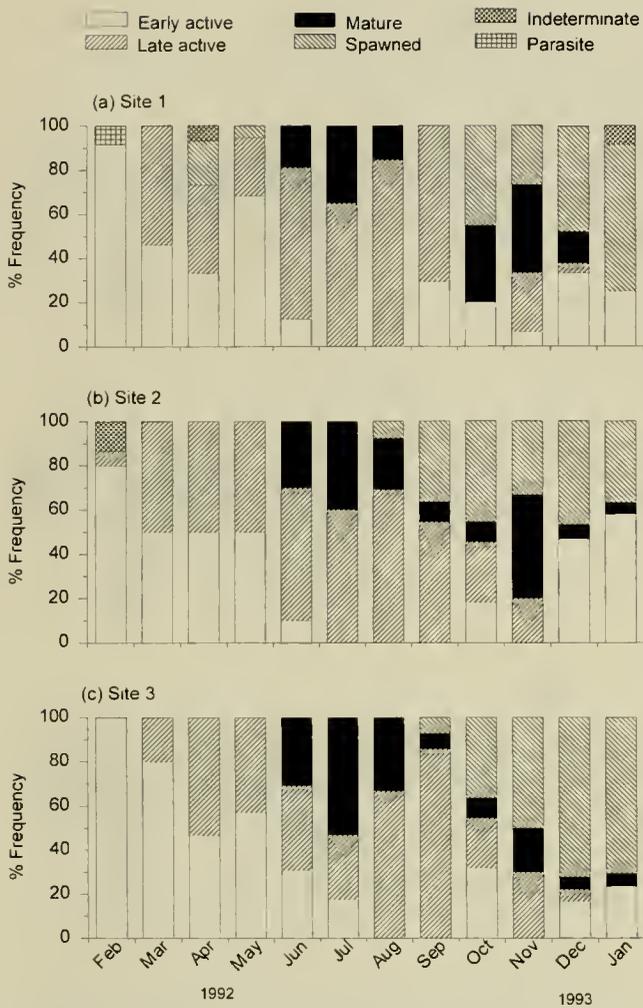


Figure 4. Proportional bar chart of the histological data showing the reproductive cycle of female pipi at each of the three sampling sites in the Whangateau Harbour.

sites had the same general pattern of development (Figs. 7 and 8). The major difference, as with the histological study, was that site 1 matured later than sites 2 and 3. The visual appearance of females seemed to mirror the histological pattern better than males, as there were many months when mature male pipi were not identified (Figs. 7 and 8). The correlation of the visual index and the histological analysis showed a poor fit ( $r_s = 0.3054, p < 0.05$ ), but was statistically significant.

There was poor recognition of mature stages in the visual index and classified the majority of animals into the developing category (Fig. 6b, Table 1). This had the effect of increasing the numbers of correctly identified developing individuals (73%) and may account for the statistically significant correlation. Animals histologically identified as mature were sometimes (8%) visually assessed as immature although the opposite, where samples were histologically identified as immature and visually assessed as mature, was rare (2%) (Table 1). These trends were repeated at all sites. Generally there was a higher percent of females than males that were correctly visually identified when compared to histological analysis with the exception being developing males at site 2 (Table 2).

Eleven individuals out of a total of 1260 (0.87%) were found to be infected with a trematode parasite. Most of these individuals

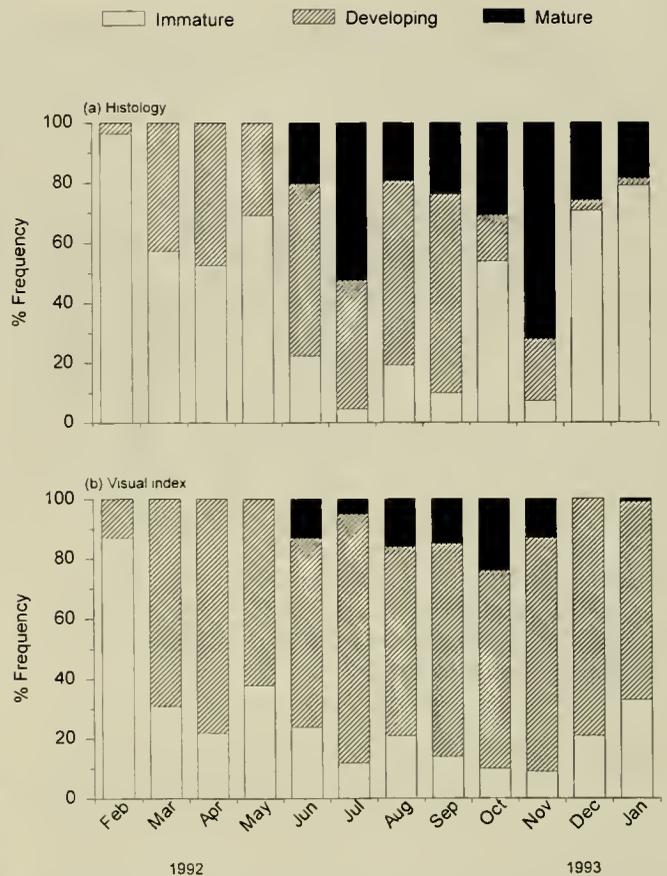


**Figure 5.** Proportional bar chart of the histological data showing the reproductive cycle of male pipi at each of the three sampling sites in the Whangateau Harbour.

had been castrated by the fluke and were not able to be sexed. There was no obvious seasonal pattern of infection with infected individuals occurring in February, March, April, July, August, September and November (Fig. 3). The number of parasites was constant between sites with the parasite occurring at all sites in low frequency (Figs. 4 and 5).

**Sex Ratio**

Of 1080 pipi examined, 528 were males and 539 females. The remaining 13 could not be sexed, due to either the trematode parasite or alternatively, the absence of sex cells in some stage five individuals. There was no significant difference in the numbers of males and females present ( $\chi^2 = 0.113, p > 0.05$ ). All three sites had statistically equal numbers of male and female pipi (Table 3a). Site 1 had more males than females, site 2 had more females than males and site three had very similar numbers of each sex. Monthly comparisons showed no statistical differences in the numbers of males and females (Table 3b). Seven out of the twelve months had more females than males and the remaining five months had more male than female pipi. Samples of the thirty pipi collected at each site each month revealed that there were three occasions in which the sex ratio was not statistically different to



**Figure 6.** Proportional bar chart of the reproductive cycle of all sites, male and female pipi combined at each of the three sampling sites. (a) The histological data using only the first three stages. (b) The visual index data.

1:1, they were in February and October at site 3 and December at site 1. All pipi sampled were dioecious and no evidence of hermaphroditism was found (Hooker and Creese 1995a).

**DISCUSSION**

One of the main benefits of using the visual index was the ability to quickly assess whether an individual pipi was mature. This might be useful for obtaining mature animals, from the wild, for spawning and larval-rearing experiments. If a large enough sample is collected, as was done in this paper, a reasonably accurate summary of the reproductive cycle can be obtained for surprisingly little effort compared to the enormous cost, in terms of time and equipment, of the standard histological technique. If a more in-depth understanding is required (e.g., sex ratios, parasite identification, description of gametogenic process), then histological analysis would be essential. Although the correlation between the histological analysis and the visual index showed a statistically significant correlation, it would be of little use in confirming the maturity of an individual. The statistical significance is likely to be an artifact of the visual index, classifying most individuals as "developing," and the large sample size. There was only a 20% chance that an individual was histologically mature when it looked visually mature. Therefore the visual index was of limited value in accurately assessing the maturity of an individual. In fact, it may even give the wrong result if used to answer specific questions on the reproductive cycle of pipi.

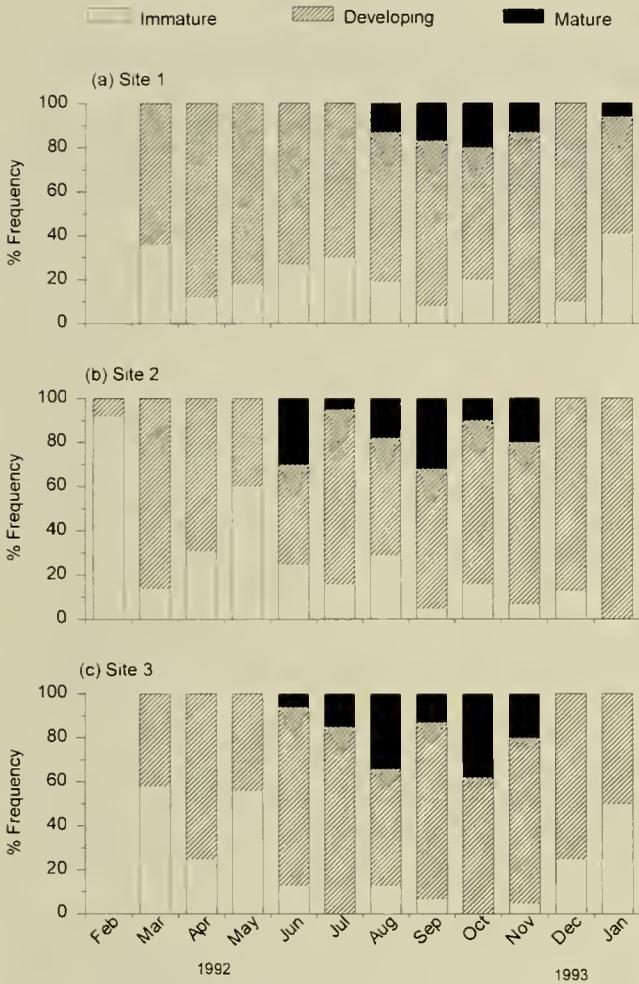


Figure 7. Proportional bar chart of the visual index data, showing the reproductive cycle of female pipi at each of the three sampling sites in the Whangateau Harbour.

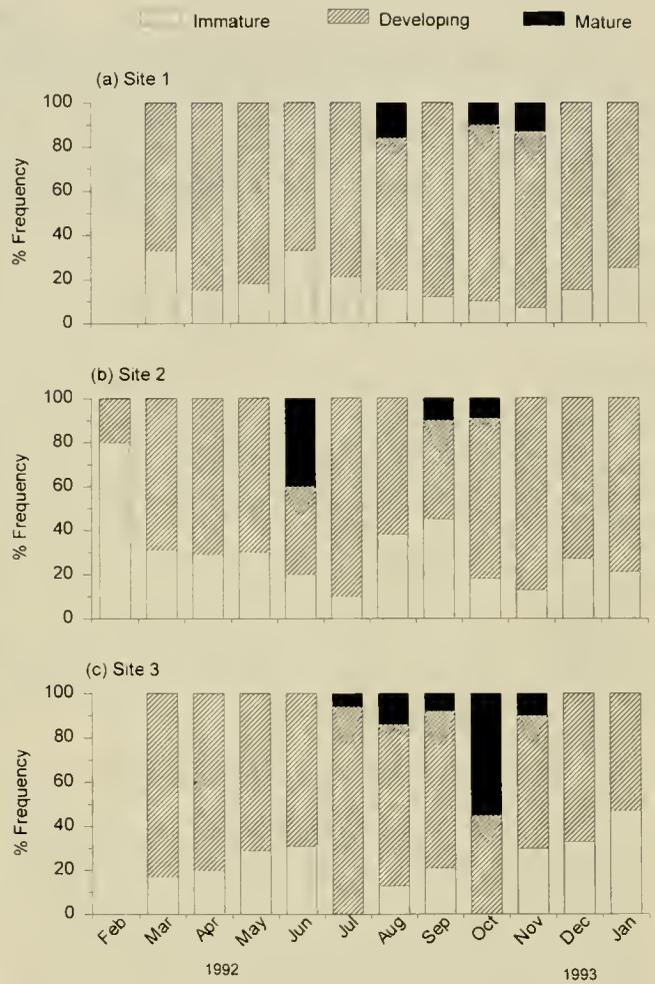


Figure 8. Proportional bar chart of the visual index data, showing the reproductive cycle of male pipi at each of the three sampling sites in the Whangateau Harbour.

Macroscopic examination of scallop gonads has been successful in determining the reproductive condition (e.g., Mason 1958, Sanders and Lester 1981). This is mainly due to the exposed and relatively separate nature of scallop gonads. This is not the case in most clams. Macroscopic examination of the gonads of clams has been used to assess the reproductive state of clams although few have formally tested the reliability of the technique (Caddy 1967, Gilbert 1978, McCreer 1983, Baron 1992). Porter (1964) noted a gross gonad condition of *M. mercenaria* and concluded that it was not reliable except in extreme conditions, which in effect is the result found in this study.

Dickie (1986) describes a spent stage undergoing phagocytosis of sex cells in pipi from Whangarei Harbour (55 km north of the Whangateau Harbour). Photographs of this phase, which are included in his report, look remarkably similar to the digenetic trematode parasite found in the present study. No resorption of sex cells was documented at any stage in the present study. A very low level of parasitic infection was found in this study of pipi from the Whangateau Harbour and was similar at all sites sampled. A single sample of thirty pipi collected intertidally in the Whangarei Harbour in September 1992 showed that 3 (10%) of the pipi were infected with the digenetic trematode parasite, which is a much greater level of parasitic infection found in the Whangateau Har-

bour. Dickie (1986) gives no indication of the numbers of the resorption stage individuals he found in his study but it is presumed to be reasonably high since it was included in a photograph. If this stage of his was, in fact, a misrepresentation of a parasitised gonad, this would suggest that levels of parasitism in pipi in the Whangarei Harbour may be consistently high. Further investigation of regional differences in the incidence of parasites is warranted, as parasitism has the potential to be ecologically important to pipi populations since it totally castrates its victims.

Sexual dimorphism is minimal in animals that shed their gametes (Strathmann 1990). Bivalves lack external characteristics for determination of sex (Ropes et al. 1984). The colour of surf clam gonads is not always consistent and care should be taken when determining the sex from gonad colour (Ropes 1968). *Macoma balthica* can apparently be easily sexed by the colour of the gonad with ovaries being grey to grey-orange and testes white (Gilbert 1978). Dickie (1986) noted that the sex of pipi may be distinguished when mature by observation of the colour of the gonad, with males being slightly orange and females cream. The opposite was true in this study. Only the gonads of very mature individuals could be seen macroscopically, and when checked under the microscope, females were pink and the males cream. This is the same result as was found for *Spisula solidissima* (Schechter

TABLE 1.

Comparison of the percentages identified by the visual index of each stage (mature, developing and immature) compared to the histological analysis at each site for males and females combined.

	Mature		Developing		Immature	
	N	Percentages	N	Percentages	N	Percentages
				<b>Site 1</b>		
Mature	69	<b>19</b>	85	11	129	5
Developing		75		<b>73</b>		66
Immature		6		16		<b>29</b>
				<b>Site 2</b>		
Mature	78	<b>18</b>	107	8	136	2
Developing		68		<b>67</b>		58
Immature		14		24		<b>40</b>
				<b>Site 3</b>		
Mature	43	<b>26</b>	113	1	117	1
Developing		72		<b>49</b>		74
Immature		2		20		<b>25</b>
				<b>Total</b>		
Mature	190	<b>20</b>	305	6	382	2
Developing		72		<b>73</b>		66
Immature		8		21		<b>32</b>

Histological stages are shown along the top of the table and visual stages are shown down the left hand side of the table. Bold values signify the percentages when comparing the same stage.

1941, Sephton 1987). Microscopic analysis is the only reliable method of determining sex in pipi.

The sex ratio was not significantly different from 1:1 for pipi in the Whangateau Harbour, confirming preliminary findings by Dickie (1986) for pipi in the Whangarei Harbour (153 males and 156 females out of 309 individuals). Dickie (1986) however, found a 1:3 bias at individual sites but this may be attributable to the low sample size. Studies on other species in the genus *Paphies* have also shown a 1:1 sex ratio (Dawson 1954 and Grant 1994 for *Paphies subtriangulata*; Redfearn 1974 for *Paphies ventricosa*).

TABLE 2.

Comparison of the percentages that the visual index identified of each stage (mature, developing and immature) compared to the histological analysis for female and male pipis at each site.

Sex	Mature	Developing	Immature
	Visual Index (percentages)	Visual Index (percentages)	Visual Index (percentages)
		<b>Site 1</b>	
Female	21	75	40
Male	16	71	15
		<b>Site 2</b>	
Female	21	64	42
Male	10	71	37
		<b>Site 3</b>	
Female	44	81	24
Male	15	77	24
		<b>Total</b>	
Female	24	72	36
Male	13	74	25

Histological stages are shown along the top of the table and visual stages are shown down the left hand side of the table.

Adult clams rarely show unequal sex ratio except for hermaphroditic species (Eversole 1989). It is extremely unlikely that pipi are hermaphroditic as none was found either in the present study, or when a full-size range of animals was examined (Hooker and Creese 1995a).

Pipi do not have a well-defined reproductive cycle (Hooker and Creese 1995a). Spawning in the pipi population was not a discrete event but a series of partial spawnings of the population, over weeks or possibly months probably dependent on local environmental influences (Hooker and Creese 1995a). This mode of reproduction makes precise prediction of spawning events, or of subsequent recruitment pulses, very difficult. The overall pattern of reproductive cycle for pipi in the Whangateau Harbour was similar at all three sites sampled. The population was developing over the autumn and early winter with most individuals reaching maturity by July. From that time there was a pattern of periodic spawning over the spring and summer months. An extended spawning season from June to January was apparent at all sites, but there were subtle differences observed in reproductive timing. Reproductive cycles of several clams have been shown to vary with latitude (Porter 1964, Ropes and Stickney 1965), but there are few studies on small-scale (<1 km) variations in reproductive cycle. Bourne and Quayle (1970) and Mann (1982) combined different sites to describe the reproductive cycle of clams. Bourne and Quayle (1970) comment on the variability of the time of spawning of razor clams (*Siliqua patula*) and attribute this to the samples being collected over a wide stretch of the beach. They also state that similar studies on butter clams (*Saxidomus giganteus*) have shown that there can be considerable variation in the time of spawning in animals separated by only a few meters on a beach. Mann (1982) found minor differences in gonadal development of *Arctica islandica* despite inshore sites being consistently warmer than offshore deeper sites. He concluded that there was probably a difference between sites but it was masked by the methodology he used to describe the gonadal development. Breed-

TABLE 3.

Numbers of male, female and indeterminate pipis at each site for (a) each of the three sites and (b) monthly from February 1992 to January 1993.

(a)

	Males	Females	Indeterminate	Total	$\chi^2$ Value
Site 1	193	162	5	360	2.701
Site 2	161	196	3	360	3.431
Site 3	174	181	5	360	0.138
Total	528	539	13	1080	0.113

(b)

Months	Males	Females	Indeterminate	Total	$\chi^2$ Value
February 1992	36	52	2	90	2.909
March 1992	37	53	0	90	2.844
April 1992	48	39	3	90	0.931
May 1992	43	47	0	90	0.178
June 1992	39	50	1	90	1.360
July 1992	47	42	1	90	0.281
August 1992	41	48	1	90	0.551
September 1992	42	46	2	90	0.181
October 1992	53	37	0	90	2.844
November 1992	40	50	0	90	1.111
December 1992	54	36	0	90	3.600
January 1993	48	39	3	90	0.931
Total	528	539	13	1080	0.113

The critical value for  $\chi^2$  goodness of fit test of equal numbers of male and females, (1 *df*) at 95% significance is 3.841.

Willeke and Hancock (1980) found similar results to those recorded here with the spawning cycles of the gaper clam (*Tresus capax*) synchronous at the four sampling sites. They also found no difference in the spawning time between intertidal or subtidal populations. Keck et al. (1975) attributed differences in reproduction between geographic areas of hard clams (*M. mercenaria*) to environmental factors and that different physiological races existed between these areas. Dickie (1986) did not reach any conclusion regarding the differences in reproductive cycle of pipi between three intertidal sites and one subtidal site, over approximately four kilometers, in the Whangarei Harbour. His results indicate a pattern very similar to what was found in this study in the Whangateau Harbour of a synchronized reproductive cycle at the sites sampled. Subtle differences in the reproductive cycle of pipi between the sites in this study is probably due to slight variations in environmental factors at each site or alternatively, but less likely, may be attributable to the histological method used. The results in this study indicate that there was a subtle difference in the reproductive pattern between the harbour entrance site (site 1) and the

other two sites. This may be a manifestation of the higher stress environment (substantially higher densities of pipi, higher water current velocities and a shallow mobile shell substrate) experienced at the harbour entrance compared to the sites further up the harbor (sites 2 and 3). These topographical differences between site 1 and the other two sites further up the harbour may tie in with other features of the population dynamics of pipi which will be described elsewhere (Hooker and Creese 1995b, c).

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## AQUACULTURAL PRODUCTION OF NORTHERN QUAHOGS, *MERCENARIA MERCENARIA* (LINNAEUS, 1758): HIGH WATER TEMPERATURES IN THE NURSERY AND GROWTH PENALTIES OF PREDATOR CONTROL BY GRAVEL

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**ABSTRACT** Despite water temperatures in the first month of 25–33°C (average 29), shore-based nursery culture of seed hard clams (*Mercenaria mercenaria*) was conducted from mid-August for 77–102 days in central North Carolina. Average survival in this passive upweller operation was never less than 87% in any of 3 years as clams were grown to a length of 10 mm. Growth and survival of seed clams in the upwellers exhibited little or no sensitivity to experimental variation in stocking density under the densities, flow rates, and natural food concentrations used, implying that relatively high water temperatures did not induce hypersensitivity to stresses of crowding in the upwellers. In one year, the seed clams emerging from the nursery were employed in an experimental planting program for grow-out in the field. Clam survivorship at high stocking density (8800 seed clams per 3.34 m<sup>2</sup> plot) in natural sand substrate was less than one third (12 vs. 38–40%) of what was achieved either by using low stocking density (2200 seed clams per plot) or by adding 2 cm of gravel to clams at the high density. Although addition of a gravel layer provided protection from crab predation that equaled the protection provided by low density stocking, only 8% of surviving clams in the high-density plots with gravel had reached the legally marketable size of 2.5 cm thickness after 36 months, as compared to 46% in low-density plots. Because the high-density plots in natural sand exhibited an intermediate 29% of surviving clams achieving marketable size, the cause of the slower growth in the high-density plots with gravel appears to be a combined effect of food depletion at high density and perhaps also a direct or indirect interference effect of the gravel. Thus, use of gravel to protect seed clams from crab predation while maintaining high planting densities in grow-out plots may carry with it the undesirable effect of reducing growth, thereby increasing time required for return of investment and increasing total clam mortality because of longer exposure to risks in the field. Nevertheless, our successful nursery culture of hard clams in relatively warm water temperatures from mid-summer into autumn in central North Carolina implies that clam aquaculturists may be able to control costs by purchasing smaller low-cost seed clams and growing them to planting size even using warm estuarine waters of the southeast.

**KEY WORDS:** Growth, high temperature, *Mercenaria mercenaria*, planting density, predator control, survival, upwellers

### INTRODUCTION

For many years, there has been great interest in developing commercially viable aquaculture methodologies for the hard clam, *Mercenaria mercenaria* (Castagna and Kraeuter 1981). Landings of this species in the traditional exploitation fisheries have been declining, while demand has remained strong and prices high. Furthermore, many states allow leasing of estuarine bottom for aquaculture operations, thereby promoting enhanced production through culture programs. Unlike the American oyster (*Crassostrea virginica*, Gmelin), the hard clam does not suffer the problems of high losses to parasitic diseases, such as the protozoans MSX and "Dermo," and has a far more robust physiology than scallops, which require constantly high salinities, excellent flushing, and low turbidity. The hard shell of hard clams renders handling, harvest, and shipping less injurious and more efficient than what is required for soft-shelled clams (*Mya arenaria*, Linné).

The primary impediment to widespread establishment of hard clam aquaculture operations has been overcoming the high natural mortalities that these clams suffer from predators (Menzel et al. 1976). Hard clams fall prey to an impressive diversity of consumers: crustaceans such as several crabs and shrimps; gastropod molluscs such as whelks, drills, and moon snails; echinoderms, namely seastars; rays and other fishes; and birds including oyster-catchers and gulls (MacKenzie 1977). Many of these predators are easily thwarted because they take only a narrow size range of prey

(snapping shrimp: Beal 1983), can be controlled by cages (larger fishes), are precluded from access to deeper water (gulls: Peterson et al. 1989), or can be successfully fenced off (whelks: Peterson 1982a). The predators least controllable remain crabs, especially blue crabs. Crabs are extremely mobile, have voracious appetites for clams, and readily invade cages and other containers by recruiting as larvae through the plankton (Whetstone and Eversole 1978). Several techniques have been developed to minimize crab predation, including application of coarse substrate to inhibit foraging effectiveness (Castagna and Kraeuter 1977), caging to exclude larger crabs from the bottom (Manzi et al. 1980), introduction of toadfish to consume invading crabs (Gibbons and Castagna 1985), and combinations of various methods (e.g., Kraeuter and Castagna 1980). Despite the partial effectiveness of these control measures, development of a site-specific predation prevention scheme remains the most critical determinant of success in hard clam aquaculture.

Hard clam aquaculture can be separated into three operational phases (Castagna and Kraeuter 1981). During the hatchery phase, adult clams are spawned and larvae fed and raised until settlement onto the substratum. During the nursery phase, the newly settled clams are grown to some larger size in a laboratory system, usually of raceways or upwellers. During the final grow-out phase, seed clams taken from the nursery are introduced into a field environment where they exploit naturally available foods to grow to marketable size. Because of escalating demands for space and added

water flow (food flux), clams become increasingly costly to grow to larger sizes in a nursery (Castagna 1984). Consequently, the purchase price for seed clams rises rapidly with increasing clam size. Aquaculturists who intend to participate in the grow-out of seed clams are thus faced with the decision of what size to purchase. The smaller seed clams come at lower cost, but the lower risk of loss to predatory crabs for larger, more expensive clams may more than compensate for the cost differential.

Many aquaculturists have land access to water of sufficient quality to permit them to construct a nursery facility of their own. This would permit purchase of very small, inexpensive seed clams, which can then be grown under predator-free conditions in the nursery to sizes appropriate for planting in the field (Castagna 1984). A reasonable strategy for hard clam culture in areas of relatively mild winters (such as in the south Atlantic and Gulf states) would be to terminate the nursery phase and introduce seed clams into the field around the end of autumn (Eldridge et al. 1979, Peterson 1990). This strategy permits the culturist to exploit the opportunities for substantial winter growth in these areas (e.g., Peterson and Fegley 1986) during the season when crabs are inactive so that the seed clams will have become much larger by spring when predation risk rises again.

To pursue this strategy requires that seed clams be held in a nursery during autumn and perhaps even some of summer, when water temperatures are at their highest. In the southeast coastal regions of North America, late summer water temperatures (e.g., Sutherland and Karlson 1977) in the estuaries commonly exceed the reported optimum of 20°C and often even the reported maximum of 31°C for hard clam growth and survival (Ansell 1968). Consequently, we conducted the study reported here as a feasibility test of conducting successful nursery operations for seed hard clams in the southeast during the season of highest water temperatures. We also manipulated clam density in the upweller nursery system to address the concern that success may depend critically upon seed clam density if the clams are already severely stressed by high temperature, as implied by the results of Manzi et al. (1986), consistent with the usual assumption that physiological stresses interact (Peterson and Black 1988). Finally, we followed the fate of these seed clams during the grow-out phase, for which we also varied planting density to assess whether any delayed physiological effects of high temperature development in the nursery would be expressed by greater sensitivity to density variation during grow-out. In these grow-out trials, we also experimentally manipulated the caging design and substrate along with seed clam density to evaluate their effectiveness in reducing predatory mortality and whether trade-offs between survivorship and growth exist to complicate the choice of predator protection strategy.

## MATERIALS AND METHODS

To assess the feasibility of establishing nursery operations for hard clam (*M. mercenaria*) aquaculture in the southeast United States during the late summer and autumn seasons when water temperatures can be relatively high, we conducted trials in an upweller system in each of three successive years, 1985, 1986, and 1987. In 1985, the trials represented a pilot study in which the only treatments compared in the upwellers were the sources of seed clams (purchased at average shell lengths of 3.7 to 4.0 mm from a Massachusetts vs. a Delaware hatchery). In 1986, trials involved a contrast of different stocking densities in the upwellers, with densities differing by a factor of 1.5 (6500 vs. 9790 clams of

average 7.4 mm length per upweller). At the end of the nursery phase, these seed clams raised in 1986 were subsequently monitored during the grow-out phase as a function of planting density (2200 vs. 8800 in 3.34 m<sup>2</sup> field plots). In 1987, stocking density was again varied but over a wider range (a factor of 2.0, from 6200 to 12440 4.4 mm-long seed clams per upweller), water temperatures were recorded 2–4 times daily (early morning and late afternoon every day to measure the expected minimum and maximum respectively with some supplemental readings in midday and/or late evening) for the first month when temperatures were at their peak, and average growth rate was measured during that first warm month as well as for the entire nursery phase. Seed clams used in 1986 and 1987 came from the Wachapreague, Virginia hatchery.

All the trials described in this study were conducted in Smyrna, in Carteret County, North Carolina. The nursery operation was situated on the shore of Middens Creek, a tributary of Core Sound about 6 km from Barden Inlet, which connects Core Sound to the Atlantic Ocean at Cape Lookout. The grow-out trials were conducted on an intertidal shellfish lease near the mouth of the same creek, not far from where it enters Core Sound. The creek is shallow, with an average depth at low tide of about 0.3 m, so water temperatures are greatly influenced by atmospheric conditions. This protected nursery site at the site of Hooper Family Seafoods is therefore typical of the sort of locality available to aquaculturists in the southeast for potential use as shellfish nurseries.

The nursery facility used for this study employed a passive upweller design, in which plastic buckets with mesh bottom were used to hold the seed clams within a holding tank (Manzi et al. 1984, Manzi and Hadley 1988). Water flow was created by allowing overflow only from drilled openings near the tops of the buckets, thereby forcing water to move upward from the bottoms to the tops of the buckets. Buckets used were inexpensive 5-gallon plastic pickle buckets, 4 replicates for each treatment in 1985 and 6 for each treatment in 1986 and 1987. The holding tanks were actually tanks designed for shedding crabs, reused in the hatchery operations after the end of the soft crab season. Flows were measured periodically during the use of these upwellers, averaging 15 l min<sup>-1</sup>, which means that the contents in a bucket were being replaced every 1.26 min, on average. These flow rates provided an initial flow of 119 l min<sup>-1</sup> kg<sup>-1</sup> of clams in the low density treatment and 50 l min<sup>-1</sup> kg<sup>-1</sup> in the high density treatment in 1987 when treatment densities varied the most. By the end of the nursery phase in 1987, clam growth was sufficient to have reduced flow rates to 7.2–3.6 l min<sup>-1</sup> kg<sup>-1</sup>. These values compare to recommended flows of 23–30 l min<sup>-1</sup> kg<sup>-1</sup> (Manzi et al. 1984). Water was pumped directly from Middens Creek from a depth of about 1 m.

Procedures for data collection and maintenance of the upweller experiments were similar in each of the 3 years. Initially (5 September in 1985, 15 September in 1986, and 18 August in 1987), abundances of live clams were estimated by weight. A random sample of the newly obtained seed clams was split into four equal subsamples by coning and quartering. Each separate subsample was then spun on a salad spinner to remove excess water, weighed, and the clams counted to permit calculation of the initial weight-per-clam. That average weight-per-clam was then used to calculate the weight required to achieve the desired stocking density for each upweller bucket. A random sample of live clams was taken to allow estimation of average ( $\pm$ SE) length to the nearest 0.1 mm using vernier calipers (Table 1). At the end of the nursery

TABLE 1.

Numbers of seed clams subsampled for estimating mean length at each sampling date for each of the three years of upweller trials.

Year	Treatment	
	Delaware Clams	Massachusetts Clams
1985		
On 5 Sept start	83	84
On 16 Dec end	100	100
	Low Density	High Density
1986		
On 15 Sept start	83	84
On 2 Dec end	941	912
1987		
On 18 Aug start	208	199
On 15 Sept	869	1023
On 3 Nov end	1304	1559

phase (16 December in 1985, 2 December in 1986, and 3 November in 1987), as well as after the first month in 1987, during which daily temperature readings had been made, a random sample of seed clams was taken from each upweller after thorough mixing of all clams. That sample was again spun to remove water, and then weighed, whereupon the clams were counted, assayed for mortality, and measured with the calipers. From these measurements, we estimated the average final length of the seed clams, the average proportion surviving, and the final wet weight biomass of clams. Sediments were cleaned out of the buckets twice each week.

The follow-up experiment designed to evaluate how well seed clams raised under relatively high nursery temperatures survived during the grow-out phase utilized several different techniques to minimize loss to predators. All plots (1.8 m by 1.8 m) were covered by a top made of 6 mm polypropylene mesh sewn onto a square edge made of 12 mm-diameter steel rebar poles, which was staked to the bottom. The top was not stretched taut but rather was left loose ballooning up somewhat above the sediment surface. Half of the plots were given mesh bottoms made from the same

material. These plots with bottoms were excavated to a depth of about 15 cm, lined with the mesh and then refilled with sediments before planting of seed clams. This bottom-type treatment was then crossed in a factorial design with a clam density-gravel treatment. One third of the plots with bottoms and of those without bottoms received low clam density (2200 seed clams) with no gravel; one third high clam density (8800 seed clams) with no gravel; and one third high clam density with gravel. Our density range of 660 m<sup>-2</sup> to 2635 m<sup>-2</sup> was high relative to Castagna's (1984) recommendation of 250 to 1000 m<sup>-2</sup> for 8 mm seed clams. All predator protection treatments were randomly assigned to plots, with 2–3 replicates of each treatment combination. The plots with gravel were provided a 5 cm layer of 0.33 cm<sup>3</sup> gravel spread onto the surface. Clams used to stock this experiment were taken from the upwellers on 9 December 1986 and thoroughly mixed to homogenize any effects of density treatment in the upwellers before introduction to the field grow-out plots. Plots were excavated completely on 21–22 August 1989, when surviving clams were counted and the percentage of legally harvestable (>1 inch thick) clams determined by passing them through grader grids.

## RESULTS

The seed hard clams grew and survived well during the nursery phase in each of the 3 years of these trials (Table 2). Estimated survivorship was no lower than 87% for any treatment in any year for the 77–102 days in the nursery. There was no significant difference (at  $\alpha = 0.05$  in a t-test) between any pair of density treatments during any year in average clam survivorship. The average survivorship of the Delaware clams was significantly higher ( $p < 0.05$ ) than that of the Massachusetts clams, but the difference between 93 and 87% was small. Average shell length of surviving seed clams was about 10 mm for every treatment in every year, with the exception of the Delaware clams in 1985, which averaged only 7.8 mm in final length, significantly less ( $p < 0.05$ ) than the Massachusetts clams used in that year (Table 2). In the 2 subsequent years, t-tests revealed that stocking density had no significant effect on final size achieved by seed clams in these experiments. At the end of the first month of growth in the 1987 trial, however, the average size of seed clams in the low-density treat-

TABLE 2.

Growth and survivorship of seed hard clams in upwellers during late summer and autumn of three years.

	Year and Treatment					
	1985 <sup>a</sup>		1986 <sup>b</sup>		1987 <sup>c</sup>	
	Delaware	Massachusetts	Low Density	High Density	Low Density	High Density
Numbers of replicate upwellers	4	4	5	6	6	6
Average initial size ( $\pm$ SE) in mm	3.7 (0.4)	4.0 (0.3)	7.4 (0.1)	7.4 (0.1)	4.4 (0.4)	4.4 (0.4)
Average size after 1 mo ( $\pm$ SE) in mm	—	—	—	—	8.7 (0.1)	* 8.1 (0.2)
Average final size ( $\pm$ SE) in mm	7.8 (0.8)	* 10.2 (0.9)	9.7 (0.2)	9.8 (0.2)	10.3 (0.2)	9.9 (0.1)
Total days in upwellers	102	102	78	78	77	77
Average initial numbers per upweller	3000	3000	6500	9790	6220	12440
Average % survivorship ( $\pm$ SE)	93 (2.0)	* 87.3 (2.2)	106.7 (6.7)	104 (2.4)	88.6 (4.8)	87.8 (4.8)

Only three contrasts (by two-tailed t-test) revealed a statistically significant difference between treatments, as indicated by an asterisk (\* $p < 0.05$ ).

<sup>a</sup> In 1985, starting date was 5 September and termination date 16 December.

<sup>b</sup> In 1986, starting date was 15 September and termination date 2 December.

<sup>c</sup> In 1987, starting date was 18 August, first month sampling at 16 September, and termination date 3 November.

ment was slightly greater ( $p < 0.05$ ) than in the high-density treatment (Table 2).

Water temperatures measured during the first month of the 1987 trial averaged 29°C and ranged from 25–33°C (Fig. 1). Only in this third year did the nursery phase commence as early as August (Table 2). During this first month in the upwellers in 1987, average seed clam size approximately doubled and survivorship for the full 77 days was estimated to be about 88%, independent of density treatment (Table 2). If stocking density did affect growth rate during this period of presumably highest water temperatures, the effect was not detectable after the conclusion of a second month (Table 2).

Because of the absence of any effect of density on seed clam survivorship and the small-to-absent effects of density on growth of seed clams, stocking at higher clam densities produced much greater total clam production per upweller (Table 3). For every comparison of low- vs. high-density treatments in both years when stocking density was manipulated in the nursery, total final weight of clams and total clam production per upweller were substantially and significantly ( $p < 0.001$  in all t-tests except one where  $p < 0.01$ ) higher for the high-density treatment. In 1986, the clam production per upweller was 39% higher in the high-density treatments. In 1987, clam production per upweller was 65% greater in the high-density treatments during both the first, presumably warmer, month as well as during the second month (Table 3).

The results of the grow-out trials demonstrated that the addition of bottoms to plots had no effect on either survivorship or size of clams at the end of the grow-out period, but that the effects of the clam density-gravel treatments were profound. A two-factor factorial ANOVA detected no significant effect (at  $\alpha = 0.05$ ) on clam survivorship of bottoms or of the interaction between bottoms and the density-gravel factor. In contrast, there was a significant effect of the density-gravel treatment ( $p = 0.019$ ) on clam survivorship. The low-density treatment at 38% survival and the high-density treatment with gravel at 40% survival did not differ significantly from each other in SNK contrasts, but each was substantially and significantly (at  $\alpha = 0.05$ ) greater than the 12%

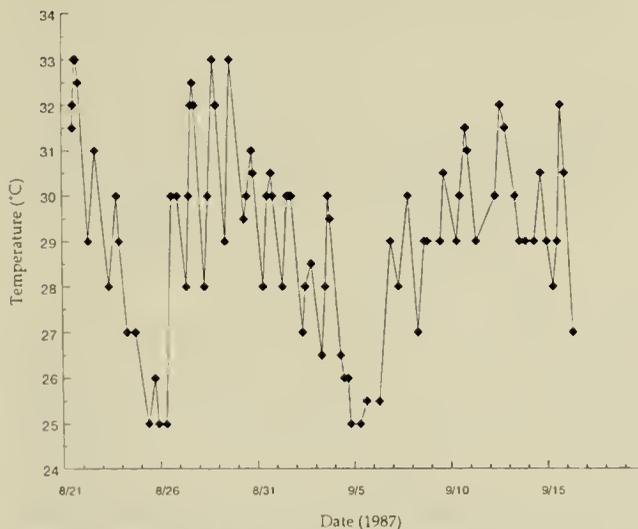


Figure 1. Water temperatures in the upwellers recorded in early morning (0800 hr) and late afternoon (1600 hr) each day with occasional added readings in midday or evening for the first month of the trials in 1987.

TABLE 3.

Total clam production per upweller in 1986 and 1987 as a function of stocking biomass.

Variable	Low Density	High Density
<b>1986</b>		
Numbers of replicate upwellers	5	6
Average total weight ( $\pm$ SE) of seed clams per upweller in gm:		
at 15 Sept start	770 (0)	1160 (0)
at 2 Dec end	2449 (90)	*** 3493 (103)
Average increase in total seed clam weight ( $\pm$ SE) per upweller in gm:	1679 (90)	*** 2333 (103)
<b>1987</b>		
Numbers of replicate upwellers	6	6
Average total weight ( $\pm$ SE) of seed clams per upweller in gm:		
at 18 Aug start	142 (0)	284 (0)
at 16 Sept	1233 (19)	*** 2082 (73)
at 3 Nov end	2372 (99)	*** 3951 (143)
Average increase in total seed clam weight per upweller in gm:		
from 18 Aug–16 Sept	1091 (19)	*** 1798 (73)
from 16 Sept–3 Nov	1139 (103)	** 1869 (80)
from 18 Aug–3 Nov	2230 (99)	*** 3667 (143)

Symbols indicate significance in a t-test (\*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

average survival of clams in the high-density treatment lacking gravel (Fig. 2).

ANOVA again revealed no significant effect (at  $\alpha = 0.05$ ) on the percentage of surviving clams that exceeded marketable size of plot bottoms or of the interaction between bottoms and the clam density-gravel treatment. However, there was a significant effect

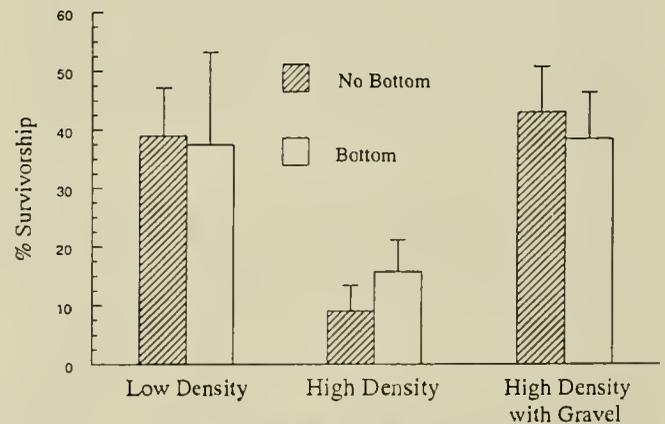


Figure 2. Average percent survivorship per plot (+SE;  $n = 2$  or  $3$ ) for seed clams planted in grow-out trials in December 1986 and recovered in December 1989, as a function of treatment. A two-factor crossed ANOVA demonstrated no significant effect of mesh bottoms on the plots and no significant interaction at  $\alpha = 0.05$  between bottoms and the clam density-gravel factor, whereas the clam density-gravel treatment was significant ( $p = 0.019$ ). SNK tests revealed that mean survivorship in the low-density and in the high-density with gravel treatments were significantly ( $p < 0.05$ ) greater than in the high-density treatment lacking gravel. Analysis was conducted on untransformed percentages because Cochran's test revealed no detectable heterogeneity of variances.

of the density-gravel treatment ( $p = 0.018$ ). SNK contrasts at  $\alpha = 0.05$  revealed that the only statistically detectable difference between treatments existed between the 46% marketable on average in low-density plots and the 8% marketable in the high-density plots with gravel added (Fig. 3). The average percent marketable of 29% observed in the high-density plots without gravel was intermediate between and indistinguishable from the other 2 means. If marketable percentage is based upon the numbers of clams planted rather than the numbers recovered, ANOVA again revealed a significant effect ( $p = 0.007$ ) of the clam density-

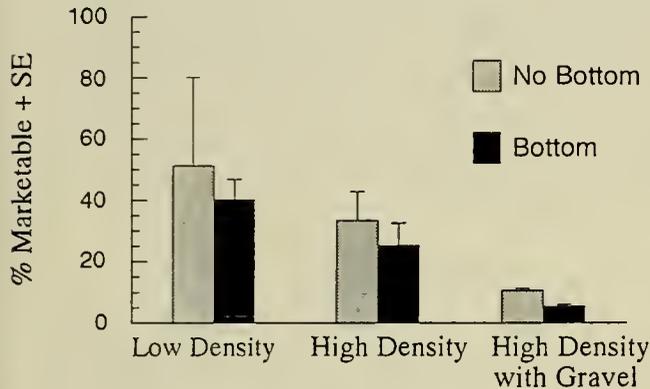
gravel factor but not of the plot bottoms or of the interaction between bottom and the density-gravel treatment. SNK showed that the average marketable percentage of planted clams was significantly greater in the low-density plots at 16% than in either the high-density or the high-density with gravel treatments, both of which exhibited return of only 3% of planted clams at marketable size by December 1989, over 3 years after planting (Fig. 3).

## DISCUSSION

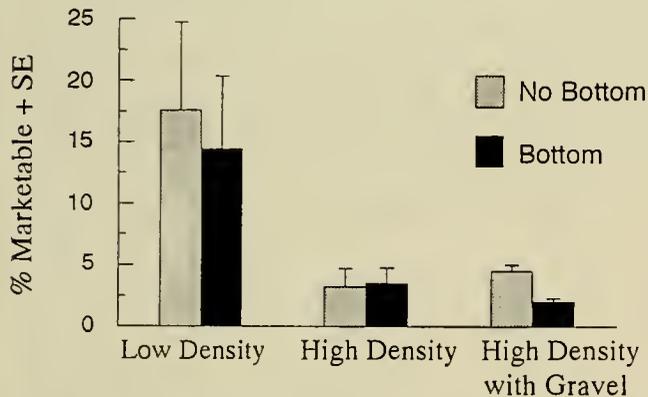
It is clear from the results of our nursery trials that juvenile hard clams do show net growth with low levels of mortality when cultured in North Carolina at water temperatures of 25–33°C. Over the range of stocking densities, under the flow conditions, and with the food concentrations in natural estuarine waters that we used, there was no detectable hypersensitivity to stress of stocking density at these relatively high water temperatures. In fact, stocking density did not detectably alter the average final size or survivorship of seed clams in either 1986 or 1987, the years when density was experimentally varied. Despite the real potential for food depletion in the nursery by these suspension-feeding bivalves (e.g., Hadley and Manzi 1984), clam production was far greater at the higher stocking densities. Water temperatures measured during our experiments match well the temperatures reported for a similar estuarine environment in this region (Beaufort, NC) over a 3-year period (Sutherland and Karlson 1977). Such temperatures exceed the reported physiological optimum of 20°C for hard clam growth and occasionally even the maximum of 31°C for growth (Ansell 1968), yet our results imply that shore-based nursery culture can still be a viable means of reducing costs of seed in hard clam aquaculture operations in the southeast United States (Castagna 1984). The negligible growth and high mortality reported by Hadley and Manzi (1984) for seed hard clams in raceway nurseries when water temperatures exceeded about 28°C for extended periods does not seem to apply universally and does not appear to inhibit late summer growth in our upweller system. Our results are more consistent with the report of Craig et al. (1988) showing growth of *M. mercenaria* in Texas bays at temperatures up to 35°C. It is possible that in some localities with more limited water exchange and on some occasions, such as in July, water temperatures may exceed those tested in our study and may prove to be an inhibition to successful nursery culture. However, we encountered no such impediment to hard clam culture using natural sea water during late summer and autumn of the 3 years of our experimental trials.

The use of seed clams grown at such relatively high water temperatures in the nursery also did not appear to reduce their subsequent survival in the grow-out phase in field plots. Under conditions of low density or high density with gravel to protect clams from predators, survivorship from planting to harvest of about 40% is in line with some of the best results achieved in North Carolina clam gardens. Growth rates on this shellfish lease were relatively slow (e.g., Peterson and Beal 1989), with at most 40% of surviving clams reaching the 1-inch thick size required for legal harvest after 36 months of grow-out in the field. The causes of such slow growth seem likely to be the intertidal elevation of this bottom lease (Peterson and Black 1987), which exposed the plot to air for extended periods of most days, and the relatively slow currents and minimal water exchange across the site. Furthermore, our density range of 660 to 2635 m<sup>-2</sup> is greater than the 250 to 100 m<sup>-2</sup> recommended for grow-out plots by Castagna

### Marketable percentage of surviving clams



### Marketable percentage of planted clams



**Figure 3.** The average percent (+SE;  $n = 2$  or  $3$ ) of surviving (top) and of initially planted (bottom) clams that reached legally marketable size (2.5 cm thickness) after 36 months in field grow-out plots, as a function of treatment. For data in both the top and bottom graphs, a two-factor crossed ANOVA demonstrated no significant effect of mesh bottoms and no significant interaction at  $\alpha = 0.05$  between bottoms and the clam density-gravel treatment, whereas the clam density-gravel treatment was significant ( $p = 0.018$  for the top graph and  $p = 0.007$  for the bottom). SNK tests at  $\alpha = 0.05$  revealed that the only significant difference in the top graph existed between the low-density and high-density with gravel treatments, while for the data in the bottom graph the low-density treatment differed significantly from both the high-density and the high-density with gravel treatments. Analysis was conducted on untransformed percentages because Cochran's tests revealed no detectable heterogeneity of variances.

(1984), which implies that food depletion contributes to the slow growth observed at the higher densities of clams.

The results of the grow-out trials do provide some new insights for the effective practice of hard clam aquaculture. It is interesting that mesh bottoms failed to add any protection against losses to predators even though the most important enemy of seed clams in this region, the blue crab, is a common burrower. Survivorship in the field plots was greatly improved by employing a low rather than a high planting density, presumably because of density dependence in the functional response of predatory crabs (Eggleston et al. 1992). A similarly high survivorship was also achievable by adding gravel to seed clams at high density, as expected from previous studies demonstrating how shell hash or gravel interferes with predation by blue crabs on seed clams (e.g., Castagna and Kraeuter 1977, Gibbons and Castagna 1985). This means of solving the problem of high potential crab predation while minimizing the area used for planting and thus minimizing costs of materials, maintenance, and harvest clearly carried with it a substantial growth penalty in the system that we studied. Although addition of gravel permitted high survivorship of clams at high planting densities, greatly reduced growth under these conditions represents a substantial economic penalty of slowing the return on investment as well as probably ultimately reducing survival because of the longer time of exposure to mortality risks in the field plots.

The reduced clam growth in this treatment appears to be a consequence not only of the higher local density, which induces food depletion and competitive reductions in growth if densities are high enough (e.g., Eldridge et al. 1979, Peterson 1982b, Peterson and Black 1987, Peterson and Beal 1989), but perhaps also of the presence of the gravel layer. It is not clear how the gravel would directly reduce the feeding success of a suspension feeder like *M. mercenaria* because, although hard clam growth has been

correlated with sediment grade (e.g., Pratt and Campbell 1956), this correlation is most likely not causal but instead a consequence of both variables responding to the physical energy regime (Grizzle and Morin 1989). Further study of the mechanisms by which the presence of a gravel layer may directly and indirectly influence hard clam growth is in order. Nevertheless, even in the absence of a mechanistic understanding of this effect, the implications for hard clam aquaculture are evident. Under conditions of our grow-out trials, gravel addition may reduce the per-day losses of seed clams to predators as much as substantially lowering planting density, but this approach carries with it large growth penalties and likely survivorship penalties associated with longer grow-out periods. These factors must enter into any complete bioeconomic analysis of the feasibility of alternative methods of hard clam aquaculture. Furthermore, our successful use of natural sea water from a North Carolina estuary to conduct hard clam nursery operations in the warm temperatures of late summer and fall provides evidence that hard clam aquaculturists in the southeast United States have another potential option to paying a hatchery for the high costs of large seed clams.

#### ACKNOWLEDGMENTS

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# GONADAL NEOPLASIA IN NORTHERN *MERCENARIA MERCENARIA* (LINNAEUS, 1758) AND SOUTHERN *M. CAMPECHIENSIS* (GMELIN, 1791) QUAHOGS AND THEIR HYBRIDS CULTURED IN SOUTH CAROLINA\*

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**ABSTRACT** *Mercenaria mercenaria*, *M. campechiensis* and their reciprocal hybrids were cultured in South Carolina. Histopathological examination revealed gonadal neoplasia in 47% of 440 quahogs sampled from September 1987 through October 1988. Severity of gonadal neoplasia exhibited a bi-phasic seasonal cycle. Maximum severity occurred May-July and September-October and minimum severity in March and August. Mean shell lengths of quahogs diagnosed with gonadal neoplasia were equal to or larger than quahogs without neoplasia. Among male, female and undifferentiated sexual classifications, neoplasia prevalence and severity was highest in the undifferentiated sex group. Prevalence and severity of gonadal neoplasia in the hybrids were greater than in either *M. mercenaria* or *M. campechiensis*. Examination of gonads from a collection of hybrids in July 1992 revealed a 95-100% prevalence and a significant increase in neoplasia severity over levels observed in hybrids from July 1988.

**KEY WORDS:** Gonadal neoplasia, bivalve molluscs, *Mercenaria*

## INTRODUCTION

Gonadal neoplasia was first reported in the northern quahog, *Mercenaria mercenaria* (Linnaeus, 1758), by Yevich and Barry (1969). They observed one case in 1300 quahogs collected in 1968 from Narragansett Bay, RI. Subsequent sampling revealed 2.4% prevalence in the quahogs from Narragansett Bay (Barry and Yevich 1972). More recently, gonadal neoplasia was found in *Mercenaria* spp. and hybrids from Indian River, Florida (Hesselman et al. 1988, Bert et al. 1993). Annual mean prevalence in the Indian River population was 11.6% (Hesselman et al. 1988), whereas those electrophoretically classified as *M. mercenaria*, *M. campechiensis* (Gmelin 1791) and hybrids was 6.5%, 11.8% and 21.6%, respectively (Bert et al. 1993). Bert et al. (1993) suggested that naturally occurring *Mercenaria* hybrids were more susceptible to neoplasia than the two species.

Hybrids of *M. mercenaria* and *M. campechiensis* were first produced by Loosanoff (1954). Evaluations of growth and survival characteristics of *Mercenaria* hybrids were done at several locations from New York to the Virgin Islands (Haven and Andrew 1957, Chestnut et al. 1957, Menzel 1964, Sunderlin et al. 1975, Manzi and Hadley, unpubl. data). Over this geographical range, average growth of hybrids equalled or exceeded the growth of the parental species. *M. mercenaria* survived better in northern latitudes whereas in southern latitudes survival of *M. campechiensis* was better and hybrid survival was generally intermediate. Subsequently, Menzel (1977) observed that other traits (e.g., shell closure) were also influenced by the hybridization of *Mercenaria*. In our laboratory a study was initiated to determine the consequences of hybridization on the gametogenic cycle. During this

study, specimens were found to have a proliferative disorder in the gonad which was identified as a germinoma (Registry of Tumors in Lower Animals Accession Number-5556).

This paper reports the results of a microscopic examination of gonads from quahogs of known pedigree to determine the prevalence and severity of neoplasia in individual pedigrees, relationships to season, sex and size of quahogs, and the progression of the disorder with age. The study was conducted to improve our understanding of neoplasia and implications for clam aquaculture.

## MATERIALS AND METHODS

### Culture and Sampling

*M. mercenaria* from Milford, CT, and *M. campechiensis* from Port St. Joe, FL, were spawned in November 1985 and January and February of 1986. Crosses included: *M. mercenaria* female × *M. mercenaria* male (MM); *M. campechiensis* female × *M. campechiensis* male (CC); *M. mercenaria* female × *M. campechiensis* male (MC); and *M. campechiensis* female × *M. mercenaria* male (CM). Individuals from spawns and crosses were held separately in recirculating downwellers at Waddell Mariculture Center until the quahogs were moved to a nursery at Folly River, South Carolina. Quahogs from the three spawns were combined by cross on 5 May 1986 and maintained in downwellers until planting (10 October 1986). Quahogs of each cross were planted in the Folly River at about 960/m<sup>2</sup> in separate protected trays. Average shell lengths (SL) at planting were 10.3 mm, 17.2 mm, 15.6 mm and 15.0 mm SL for MM, CC, MC and CM, respectively (Manzi and Hadley, unpubl. data). Samples were collected monthly from September 1987 through November 1987 and January 1988 through October 1988 and preserved in 10% buffered formalin. Quahogs were not sampled from the CC tray in February 1988 and from the MM tray in October 1987 and September 1988 because of poorer survival and lower stocking rates in these treat-

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ments. Four years later (7 July 1992) the hybrids were sampled to evaluate the progression of the neoplasia. The parental species were not available for sampling because low survival forced termination of that part of the hybridization study (Manzi and Hadley, unpubl. data).

#### Specimen Preparation

Shell length (anterior-posterior axis, SL) was measured to the nearest 0.1 mm. Gonadal tissue was dissected from the mid-lateral portion of the visceral mass, dehydrated in an alcohol series, cleared in xylene and embedded in paraplast. Sections were cut at 8–10  $\mu\text{m}$ , stained with Harris' hematoxylin and counterstained with eosin. The samples taken 7 July 1992 were processed for histopathology (Davidson's fixative) according to the procedures of Howard and Smith (1983).

#### Tissue Evaluation

Slides for each quahog gonad were microscopically examined and the sex classified as male, female or undifferentiated (Eversole et al. 1980). The portion of tissue section used to sex quahogs was searched for tight clusters of uniform cells with nuclei exhibiting marginated chromatin (Fig. 1) characteristic of the gonadal neoplasia described by Barry and Yevich (1972) and Hesselman et al. (1988). To determine severity of the neoplasia, the sections were classified into one of five categories: absent, very low, low, moderate and high according to the number of abnormal germinal cells per cluster and the number of clusters per follicle and inspected microscopic field. The very low category was characterized by the appearance of one 1 to 3 neoplastic cell clusters per microscope field whereas in the low severity category the slides had 2 to 3 clusters of 1 to 3 cells per follicle and field. Moderate severity

sections contained larger clusters ( $n = 4-6$  cells) and more clusters per follicle ( $n = 2$ ) and field ( $n = 3-5$ ). In high severity sections, greater than 6 clusters ( $n > 6$  cells) were present and more than 2 clusters were observed in follicles with more than 5 clusters in the microscope field. Severity categories from absent through high were assigned values 1 to 5 for statistical analysis.

#### Statistical Analysis

Estimates of the severity of neoplasia were based on assigned values (1–5) for individual quahogs. These severity values were treated as continuous variables. Analysis of variance (ANOVA) was used to test for differences in severity of neoplasia among classifications of sexes and crosses. Mean shell lengths within crosses were compared with ANOVA between those quahogs with and without neoplasia and among the five severity categories. Differences in prevalence (i.e., the percentage of quahogs with neoplasia) among crosses were examined by ANOVA using monthly percentages from September 1987 to October 1988 as individual observations. To test the progression of neoplasia between 1988 and 1992, the severity values and shell lengths were compared with ANOVA. Scheffe tests were used to detect differences in multiple mean tests and Tukey tests were used in paired comparisons. Alpha level was set at 0.05.

## RESULTS

Tight clusters of uniform, basophilic undifferentiated cells with nuclei exhibiting conspicuously marginated chromatin were observed in the gonads of quahogs cultured in Folly River, SC. These cells were identical to the gonadal neoplasms described in quahogs by Barry and Yevich (1972) and Hesselman et al. (1988). Clusters of neoplastic cells were associated with the germinal ep-

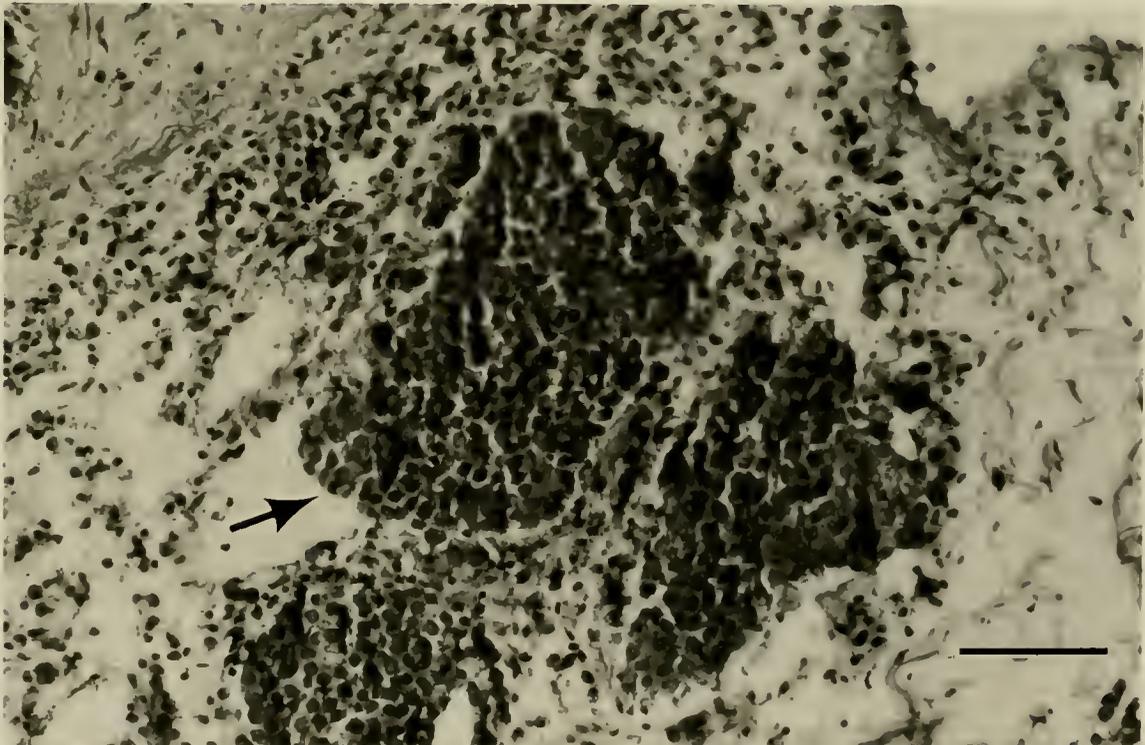


Figure 1. Representative section of the high category used to score the severity of neoplasia. Neoplastic cells (arrow) occupy nearly all of the follicle of the *M. campechiensis* female  $\times$  *M. mercenaria* male hybrid collected July 1988 from Folly River, SC. Scale bar = 50  $\mu\text{m}$ .

ithelium of the follicles or free in the follicles. In advanced cases, these cells filled the follicles (Fig. 1). Although other tissues were not specifically sampled, invasion of neoplastic cells into hepatopancreas was detected in a few specimens (Fig. 2).

Gonadal neoplasia was observed in 47% of the quahogs ( $n = 440$ ) collected from September 1987 to October 1988. Overall average severity value was 1.89. Of the quahogs with gonadal neoplasia ( $n = 206$ ), the low severity condition was the most frequent (44%) and high severity the least frequent condition (5%).

Sex composition of the September 1987–October 1988 sample was 39% male, 40% female and 21% undifferentiated (i.e., those individuals in which the sex was indeterminable because oogenic and spermatogenic cells were lacking in the sections). The prevalence of quahogs with neoplasia was higher (59%) in the undifferentiated sexual classification than in males (42%) and in females (45%) (Table 1). Severity of the disease differed significantly among sexual classifications; average severity value for undifferentiated quahogs (2.22) was the highest whereas the severity values for males (1.69) and females (1.90) were similar (Table 1). The proportion of undifferentiated quahogs (6%) rated high in severity was more than double that observed in males (<1%) and females (3%).

Quahogs examined from the September 1987–October 1988 samples ranged from 19.9–61.4 mm SL, and those with gonadal neoplasia were 20.1–51.2 mm SL. Average shell length of parental species quahogs with gonadal neoplasia was similar to those quahogs without neoplasia (Table 2). Significant differences were detected in the mean shell length among the five severity categories in hybrids. The shell lengths of the quahogs with the more advanced stages of neoplasia for MC (i.e., moderate) and CM

hybrids (i.e., high) were larger than shell lengths of hybrids without neoplasia.

Table 3 summarizes the mean prevalence and mean severity values for gonadal neoplasia by cross from September 1987 through October 1988. Average monthly prevalence of neoplasia in the CM hybrids (82.0%) was significantly greater than the CC (15.6%) and MM (32.5%) parental species. Gonadal neoplasia was more severe in the hybrids; the mean severity values for CM (2.68) and MC (1.87) hybrids were significantly higher than the parental species CC (1.41) and MM (1.13).

Gonadal neoplasia was diagnosed in all the CM hybrid monthly samples and in three months the prevalence reached 100% (Fig. 3). In fact, all the monthly prevalences ( $n = 13$ ) exceeded 50% for the CM hybrid. Neoplasia was found in 12 of 13 monthly samples of the MC hybrid, with a 77.8% maximum monthly prevalence. Infected quahogs were found in 6 of the 11 months in which the parental species MM was sampled and 7 of 12 months CC was collected. Although the highest monthly prevalence for parental species was 75%, all but 3 monthly samples contained fewer than 50% infected individuals. No obvious seasonal pattern in neoplasia prevalence was found (Fig. 3).

The monthly mean severity values for the hybrids exceeded the corresponding monthly mean values for the parental species in every month but September 1987 (Fig. 4). Overall, the higher mean severity values occurred during the months of May–July and September–October. The highest mean value for each cross was observed during one of these two periods. Lowest severity values were observed in March and August when none of the mean values exceeded 2.00 (Fig. 4).

The frequency of gonadal neoplasia in the MC and CM hybrids increased between July 1988 and July 1992 (Table 4). Correspond-

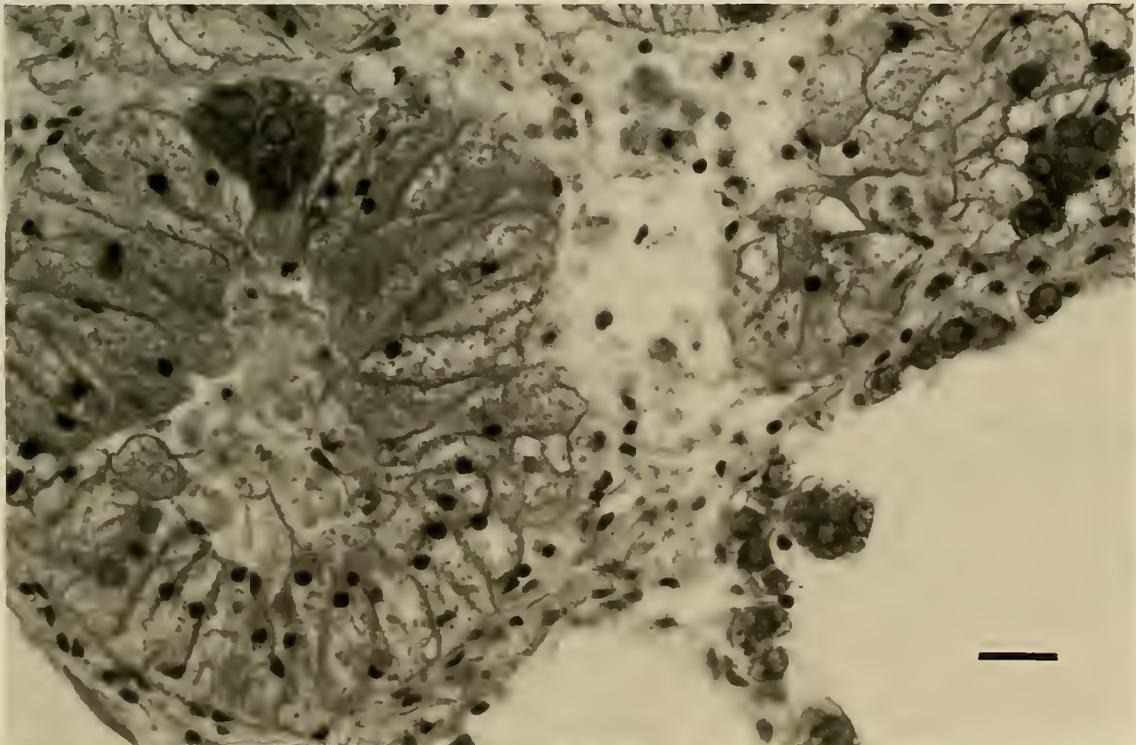


Figure 2. Neoplastic cells in hepatopancreas from *M. mercenaria* female  $\times$  *M. campechiensis* male hybrid collected July 1992 from Folly River, SC. Scale bar = 50  $\mu$ m.

TABLE 1.

Prevalence (%) and mean severity values (1–5) for *Mercenaria mercenaria* parental species (MM), *M. campechiensis* (CC), hybrids from *M. mercenaria* female × *M. campechiensis* male cross (MC) and the reciprocal cross (CM) for the September 1987–October 1988 sample period

Cross	Male			Female			Undifferentiated		
	N	Preval.	Severity	N	Preval.	Severity	N	Preval.	Severity
MM	33	15.2	1.15	28	39.3	1.54	17	58.8	1.71
CC	42	11.9	1.21	53	17.0	1.36	18	22.2	1.50
MC	55	45.5	1.75	51	47.1	2.00	14	57.1	1.86
CM	40	92.5	2.58	45	77.8	2.64	44	75.0	2.82
Total	170	42.4	1.69 <sup>a</sup>	177	45.2	1.90 <sup>a</sup>	93	59.1	2.22 <sup>b</sup>

<sup>a,b</sup> Severity values across rows not sharing the same superscript are significantly different at  $p < 0.05$ .

ing significant increases in mean severity values of neoplasia were also observed over the 4-year period. Of the quahogs with gonadal neoplasia, 84.2% of CM and 42.1% of MC specimens in July 1992 had either moderate or high rates of infection, whereas only 50% of CM and none of the MC quahogs had comparable rates in July 1988. The mean shell lengths of CM and MC quahogs used in this comparison were significantly larger in July 1992 than in July 1988.

#### DISCUSSION

The prevalence of gonadal neoplasia (47%) observed in this study was an order of magnitude higher than the <0.1% and 2.6% observed in the *M. mercenaria* from Narragansett Bay, RI (Yevich and Barry 1969, Barry and Yevich 1972) and four times that of 11.6% for *Mercenaria* spp. and hybrids from Indian River, FL (Hesselman et al. 1988, Bert et al. 1993). Although Bert et al. (1993) used a different scoring method, it appears that progression of the disorder was more advanced in the quahogs from Indian River than in South Carolina. They scored 25% of their specimens with neoplasia in the top two stages (i.e., >75% of the gonad follicles contained neoplastic cells) compared to scoring 18% of our quahogs in the moderate or high severity categories. Quahogs examined from the Indian River were larger, probably older and presumably more likely to exhibit advanced stages of gonadal neoplasia than the quahogs cultured in Folly River if the disorder was not a major source of mortality. We observed a significant increase in mean severity values and a corresponding increase in prevalence in the MC and CM hybrids from July 1988 to July 1992 (Table 4). These hybrid quahogs continued to grow and did not

experience any unusual mortality during this period (N. Hadley, S.C. Wildlife and Marine Resources Dept., personal communication).

Peters et al. (1994) identified germ cell, stromal and gonadoblastomal origins of gonadal neoplasm in a reevaluation of 178 cases of the disorder in 14 marine bivalves. Gonadal neoplasia in *Mercenaria* were exclusively of germ cell origin (Yevich and Barry 1969, Barry and Yevich 1972, Hesselman et al. 1988, Peters et al. 1994, this study). As expected, neoplastic cells were most frequently observed within the follicles, but invasion did occur to the hepatopancreas, kidney, red gland, pericardial cavity, heart, stomach, typhlosole and genital pores of *Mercenaria* (Yevich and Barry 1969, Barry and Yevich 1972, Hesselman et al. 1988, this study). Impact of gonadal neoplasia and its invasion into other tissue has yet to be experimentally determined in *Mercenaria* as has been the case with hematopoietic neoplasms in *Mya arenaria* (e.g., Leavitt et al. 1990, Brousseau and Baglivo 1991a, b). Examination of histological sections indicate that gametogenesis may be disrupted in the more advanced cases of neoplasia in *Mercenaria* (Hesselman et al. 1988, this study—Fig. 1).

The shell lengths of quahogs diagnosed with gonadal neoplasia were equal to or larger than quahogs without neoplasia (Table 2). Although neoplasia may arrest gametogenesis in the more advanced cases, it appeared not to limit growth. If reproductive functions are curtailed, then the energy normally utilized for gametogenesis would be available for growth (Eversole 1989). However, decreases in growth are expected as neoplastic cells continue to invade and disrupt other tissue functions.

Prevalence of gonadal neoplasia was similar in male (42%) and

TABLE 2.

Mean and standard deviation of quahog shell length (mm) for *Mercenaria mercenaria* (MM) parental species, *M. campechiensis* (CC), hybrids from *M. mercenaria* female × *M. campechiensis* male cross (MC) and the reciprocal cross (CM) by severity category for the September 1987–October 1988 sample period.

Cross Severity	MM			CC			MC			CM		
	N	$\bar{X}$	SD	N	$\bar{X}$	SD	N	$\bar{X}$	SD	N	$\bar{X}$	SD
Absent	52	34.52 ± 6.95		95	41.06 ± 5.33		62	42.17 ± 5.73 <sup>a</sup>		23	40.73 ± 5.09 <sup>a</sup>	
Very low	18	32.84 ± 7.78		5	40.96 ± 1.21		18	44.10 ± 6.30 <sup>ab</sup>		29	40.34 ± 5.11 <sup>a</sup>	
Low	7	34.47 ± 6.89		9	42.77 ± 3.94		22	43.25 ± 5.25 <sup>ab</sup>		44	41.04 ± 4.57 <sup>a</sup>	
Moderate				2	45.30 ± 6.45		6	47.20 ± 2.85 <sup>b</sup>		14	42.43 ± 5.94 <sup>ab</sup>	
High				2	43.05 ± 3.89		3	45.83 ± 7.48 <sup>ab</sup>		7	45.77 ± 3.88 <sup>b</sup>	
Total w/neoplasia	25	38.87 ± 7.15		18	42.58 ± 3.58		49	44.20 ± 5.57		94	41.83 ± 5.04	

<sup>a,b,ab</sup> Values down columns not sharing the same letter superscript are significantly different at  $p < 0.05$ .

TABLE 3.

Mean and standard deviation of the monthly prevalence (%) and severity values (1–5) for *Mercenaria mercenaria* parental species (MM), *M. campechiensis* (CC), hybrids from *M. mercenaria* female × *M. campechiensis* male cross (MC) and the reciprocal cross (CM) for the September 1987–October 1988 sample period.

Cross	Prevalence			Severity		
	N	$\bar{X}$	SD	N	$\bar{X}$	SD
MM	11	32.5 ± 24.6 <sup>ab</sup>		78	1.41 ± 0.65 <sup>a</sup>	
CC	12	15.6 ± 20.3 <sup>a</sup>		113	1.33 ± 0.84 <sup>a</sup>	
MC	13	47.0 ± 25.3 <sup>b</sup>		120	1.87 ± 1.08 <sup>b</sup>	
CM	13	82.0 ± 15.7 <sup>c</sup>		129	2.68 ± 1.12 <sup>c</sup>	

a,b,ab,c Values down columns not sharing the same superscript are significantly different at  $p < 0.05$ .

female quahogs (45%) in this study in contrast to the Hesselman et al. (1988) and Bert et al. (1993) studies that reported frequencies of female quahogs with the disorder twice that found in males. Highest prevalence was observed in the undifferentiated sexual classification (59%), a category not analyzed in these earlier studies. Statistical analysis also revealed an increase in neoplasia severity in the infected undifferentiated quahogs (Table 1). It is not clear why the severity and prevalence of gonadal neoplasia is elevated in the undifferentiated quahogs. One possible explanation is that it was easier to detect neoplastic cells in the empty follicles of undifferentiated individuals. It is also possible that the undifferentiated stage is a stressful segment of the gametogenic cycle and a time conducive for neoplastic cell proliferation. Undifferentiated quahogs are more frequently encountered after the rigors of spawnings and when quahogs undergo a sex change from a male to female (Eversole et al. 1980). Cellular oncogenes and tumor-

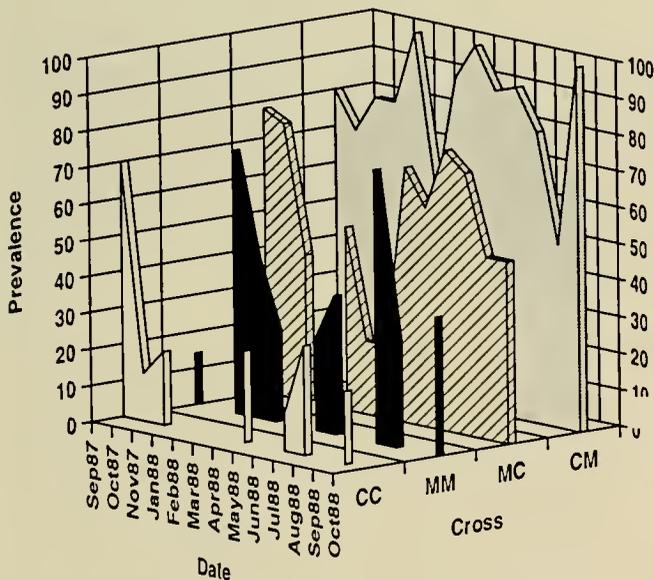


Figure 3. Prevalence (%) of gonadal neoplasia for *Mercenaria mercenaria* (MM), *M. campechiensis* (CC), hybrids from *M. mercenaria* female × *M. campechiensis* male cross (MC) and the reciprocal cross (CM) by month. Missing values represent 0% prevalence except when samples were not collected (February 1988 for CC, and October 1987 and September 1988 for MM).

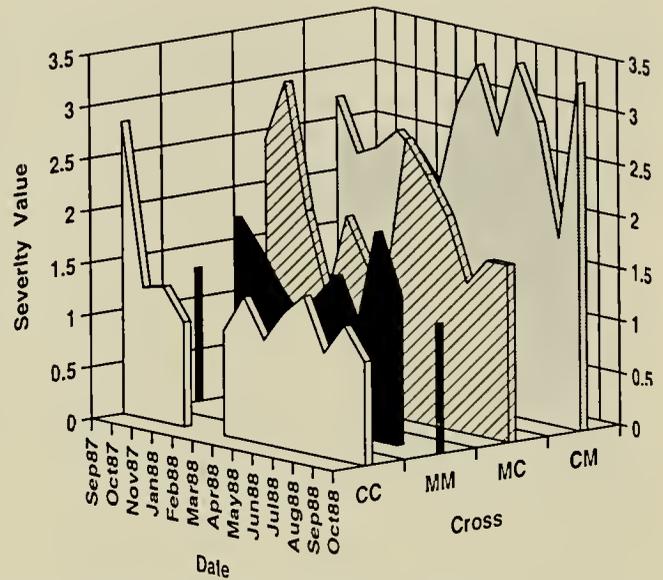


Figure 4. Mean severity (1–5) of gonadal neoplasia for *Mercenaria mercenaria* (MM), *M. campechiensis* (CC), hybrids from *M. mercenaria* female × *M. campechiensis* male cross (MC), and the reciprocal cross (CM) by month. Missing values represent no samples.

suppressor genes function to control cell growth and differentiation (Krause 1994) and may play an important role during the undifferentiated gametogenic stage.

Seasonal cycles of hematopoietic neoplasia prevalence have been reported in *M. arenaria* (Farley et al. 1986, Leavitt et al. 1990, Brousseau and Baglivo 1991b), *M. edulis* (Mix 1983), *M. balthica* (Christensen et al. 1974) and other bivalves. These studies reported both mono- and bi-phasic seasonal cycles of prevalence. Usually, a mono-phasic cycle has a peak in the colder months and minimum prevalence in the warmer months of the year whereas bi-phasic cycles have maxima in spring and fall and minima in summer and winter. The relationship between season and prevalence of gonadal neoplasia in *Mercenaria* is less clear cut. Hesselman et al. (1988) reported peak prevalence in June and the lowest prevalence in February while Bert et al. (1993) and this study found no conclusive evidence for gonadal neoplasia seasonality. However, we did observe a bi-phasic seasonal cycle of severity with two minima (March and August) and two maxima, one through the early summer (May–July) and the other in early fall

TABLE 4.

Prevalence (%), severity values (1–5), and shell length (mm) for hybrids from *Mercenaria mercenaria* female × *M. campechiensis* male cross (MC) and the reciprocal cross (CM) sampled in July 1988 and July 1992.

Cross Parameter	MC		CM	
	1988	1992	1988	1992
Number	17	20	22	19
$\bar{X}$ Shell length	42.62 <sup>a</sup>	59.69 <sup>b</sup>	43.24 <sup>a</sup>	54.43 <sup>b</sup>
Prevalence	76.50	95.00	90.90	100.00
$\bar{X}$ Severity	2.06 <sup>a</sup>	3.00 <sup>b</sup>	3.41 <sup>a</sup>	4.21 <sup>b</sup>

a,b Means within crosses not sharing the same superscript are significantly different at  $p < 0.05$ .

(September–October). These severity peaks encompassed the spawning periods for *Mercenaria* in South Carolina (Eversole et al. 1980, unpubl. data). Spawning influences the physiological status of the individual quahogs (Eversole 1989) and thereby may enhance the proliferation of neoplastic cells. The physical environment and other factors, such as mortality, can also mediate the seasonal course of neoplasia (Christensen et al. 1974, Leavitt et al. 1970, Brousseau and Baglivo 1990b).

Remission of hematopoietic neoplasia has been experimentally demonstrated in *M. arenaria* (Oprandy et al. 1981, Brousseau and Baglivo 1991a). Hesselman et al. (1988) did not observe phagocytosis, encapsulation of neoplastic cells or any other indicator of remission of gonadal neoplasia in sections of *Mercenaria*. Furthermore, our data indicate both an increase in prevalence and severity of gonadal neoplasia in *Mercenaria* hybrids between July 1988 and July 1992 (Table 4). Gonadal neoplasia appears persistent in the population of *Mercenaria* spp. and its hybrids in Florida and South Carolina.

The etiology of gonadal neoplasia in bivalves remains obscure. Farley and Sparks (1970) suggested that viruses, pollution, low salinity, genetic factors or unrecognized parasites such as protozoans and mycoplasmas may be etiologic agents. Low salinity and parasites have not been associated with neoplasia in bivalves (Farley 1977, Mix et al. 1977). Some of the neoplastic cells of *Mercenaria* contained prominent virus-like intranuclear inclusion bodies, but electron microscopy of the tissue did not confirm the presence of a virus (Hesselman et al. 1988). In addition, we noted in an ancillary experiment that gonadal neoplasia appeared not transmissible when none of the examined *M. mercenaria* ( $n = 10$ ) held in a raceway with afflicted MC and CM hybrids for 8 months developed gonadal neoplasia. The role of pollutants in the etiology of gonadal neoplasia requires more research because there are examples of affected individuals of bivalves from both polluted and relatively unpolluted environments (Yevich and Barszcz 1976, 1977, Gardner et al. 1991, Peters et al. 1994). With regard to *Mercenaria*, there is little data available to implicate pollution as an etiologic agent. The Indian River receives domestic and agricultural runoff, but the sample sites were considered unpolluted (Hesselman et al. 1988, Bert et al. 1993). Also the site used to culture quahogs in the Folly River, SC, was an approved shellfish ground in a non-agricultural area. Gonadal neoplasms have been linked to oil hydrocarbons and herbicides in bivalves, but experimental exposure-effect demonstrations are lacking (Yevich and Barszcz 1976, 1977, Mix 1986, Gardner et al. 1991, Peters et al. 1994).

Frierman and Andrews (1976) were the first to indicate a genetic basis for the occurrence of neoplasia in bivalves. Histopathological examination of *Crassostrea virginica* revealed 8.4% of two inbred lines had hematopoietic neoplasia compared to only 0.06% of other lines and native oysters collected in Virginia. Our data, with that of Bert et al. (1993), provide support for a genetic component in the etiology of bivalve neoplasia. Specifically, Bert et al. (1993) documented that prevalence of gonadal neoplasia was significantly greater in individuals electrophoretically identified as hybrids than either *M. mercenaria* or *M. campechiensis*. Using quahogs of known pedigree, we also observed that gonadal neoplasia occurred more frequently and progressed further in each hybrid than in the parental species. Recently, Van Beneden et al. (1993) successfully induced tumors in mice using DNA isolated from germinoma from the gonads of *M. arenaria* and *Mercenaria* spp. These results suggest the presence of activated oncogenes or tumor suppressor genes in bivalves and their similarity to mammalian oncogenes. Van Beneden et al. (1993) proposed that herbicide exposure was an important factor underlying oncogene activation and neoplasm development in *M. arenaria* and *Mercenaria* spp. The interaction of environmental contaminants, genetics, and other possible etiologic agents in the development of bivalve neoplasia are still not fully understood.

According to our findings and those of Bert et al. (1993), gonadal neoplasia occurs more frequently and in a more advanced stage in the hybrids than in *M. mercenaria* or *M. campechiensis*. Hybridization breeding programs to improve performance of *Mercenaria* may also increase their susceptibility to gonadal neoplasia. Because of these observations, caution needs to be exercised when promoting hybridization either through artificial breeding programs or by introducing species in new geographical ranges where natural hybridization can occur. The accompanying increase in gonadal neoplasia with hybridization of *Mercenaria* may prove detrimental to both cultured and native fisheries.

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## THE EFFECT OF SUBSTRATE DISTURBANCE AND BURIAL DEPTH ON THE VENERID CLAM, *KATELYSIA SCALARINA* (LAMARCK, 1818)

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**ABSTRACT** The cockle industry in Tasmania is currently expanding, causing concern over sustainability and over-exploitation of the state's natural stocks. Two populations of *Katelysia scalarina*, on Tasmania's east coast, were examined to determine the effects of harvesting-related substrate disturbance and burial on their mortality. Examination of harvesting methods indicated that the use of digging implements, such as a spade or pitchfork, caused significant mortality of cockles, while finger ploughing appeared to be effective and less destructive. Burial experiments indicate that adult *K. scalarina* is incapable of surviving burial at depths greater than 10 cm, but juveniles of the species appeared to be more tolerant of burial. These results have important implications for the effective management and long term stability of this fishery for the future.

**KEY WORDS:** *Katelysia scalarina*, substrate disturbance, burial depth, clams

### INTRODUCTION

The venerid genus *Katelysia* includes some of the most abundant and productive shallow water bivalves along the south coast of Australia, where they are frequently the major faunal component of shallow embayments (Wells and Roberts 1980, Coleman 1982, Roberts 1984). *Katelysia scalarina* (Lamarck 1818) is a small intertidal bivalve usually found in quiet, sheltered and sandy bays and occasionally in estuaries. In southern Australia *K. scalarina* lives in fine to medium grain sand, approximately two to four centimeters below the surface. At high tide they are covered by 0.3 to 1 m of water, depending on tidal range. *K. scalarina* appears to be restricted to the intertidal zone, where individuals are not uniformly scattered throughout the area but aggregate in groups of six or more (Nielsen 1963).

Commonly known as cockles or clams, venerids have long been recognized as a food source, while traditionally they have been inexpensive this is no longer the case. In Japan, Western Europe, and the United States of America (de Franssu 1990) certain species have become luxury foods in recent years.

In Australia, significant clam and cockle fisheries are emerging. The bulk of production in Tasmania involves *K. scalarina* (the stepped venerid) and the New Zealand venerid *Venerupis largillierti*. In southern New South Wales, *Glycymeris flammea*, a subtidal bivalve and the beach pipi, *Plebidonax deltoides*, are the major species of commercial value (Maguire 1991). Cockles in Australia are primarily harvested from natural beds, but in recent years increasing attention has been focused on clam mariculture as a means of preventing overfishing and increasing supply.

The cockle industry in Tasmania is still in its infancy, but has the potential to expand rapidly. Approximately two tonnes of cockles are currently exported to mainland markets per week, with a market value of between \$Aus3.50 and \$Aus4.50/kg, so the industry has an approximate value of \$Aus180,000 per year. Since there is a foreseeable increase in demand, concern has been expressed about sustaining and protecting naturally-occurring cockle populations and preventing the problems of over-exploitation that have occurred with other local marine organisms (e.g., orange roughy, rock lobsters).

Despite their apparent economic value, little is known about *Katelysia* spp., especially in regards to life history, so insufficient data are presently available to assess whether the clam populations

around Tasmania's coastline are capable of sustaining a significant commercial fishery. As the Tasmanian cockle industry is at an early stage it presents a rare opportunity to outline parameters for the fishery, based on research findings, before over-exploitation occurs. This study was conducted to establish whether substrate disturbance increases mortality in *K. scalarina* populations, and to determine the effects of different harvesting processes on the remaining, unharvested cockles. Burial experiments were conducted in an attempt to provide an explanation for the effect of different harvesting techniques on mortality.

### STUDY SITES

#### *Mouling Lagoon*

Mouling Lagoon is a sheltered inlet on the east coast of Tasmania (Fig. 1). The lagoon is generally less than one meter deep and is fed by two rivers and several smaller streams which may cease to flow during summer (Dec.–Feb.). The lagoon has a total water surface area of approximately 41.50 km<sup>2</sup> and a maximum tidal range between 0.8 m at the mouth and 0.3 m in its upper reaches. At low tide extensive tidal flats are exposed, with the intertidal zone extending for approximately 150 m.

#### *Pipe Clay Lagoon*

The second study site was located on the northern tidal flat of Pipe Clay Lagoon in south-eastern Tasmania (Fig. 1). The lagoon is approximately 8 km<sup>2</sup> and joins Frederick Henry Bay through a narrow tidal channel. At low tide extensive areas of tidal flats are exposed, extending for approximately 200 m. Freshwater runoff into the lagoon is minimal apart from rare periods of heavy rain, when water drains from the fringing salt marshes.

### HARVESTING EXPERIMENT

Harvesting of clams has the capacity to cause large scale disturbance of the sediment and hence the ecosystem which it supports. The usual method of digging *K. scalarina* in Tasmania is by hand, using a pitchfork to turn over the sediment, disturbing large tracts of sediment in the process. Although an efficient harvesting method, this technique has a number of potential consequences for the organisms living in the sediments (Lush 1992). The disadvantages of using digging implements to harvest soft sediment clams

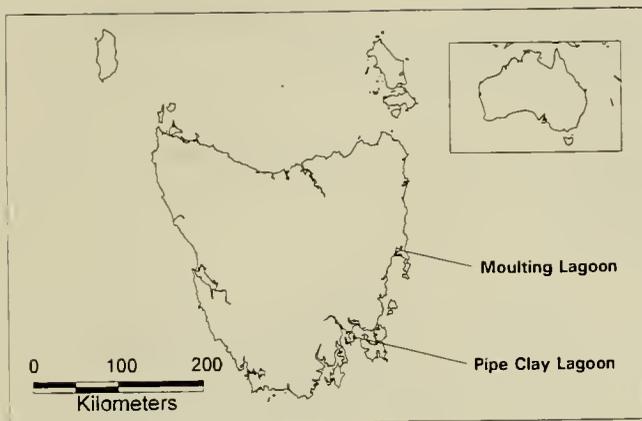


Figure 1. Map of South-East Australia and Tasmania showing study sites.

include mortality due to the incidental effects of harvesting on the clams left behind and death of pre-recruits due to burial (Needler and Ingalls 1994, Medcof and MacPhail 1964). A number of Tasmanian fishermen also utilize the finger ploughing, where the fingers are ploughed through the sediment until a cockle is encountered. This alternative harvesting method does not cause large scale disturbance and is approximately 95–100% effective (Peterson 1982).

#### Methods

Four plots, 2 × 0.5 m with 0.5 m<sup>2</sup> internal divisions, marked by steel posts, were established at the two study sites close to the low tide mark. Clams approximately 2.5–4 cm in size were collected from each of the sites and tagged. Tags consisted of a notch filed in the shell surface towards the hinge, with the opposite valve marked with ferropro superprime® paint to allow easy identification of the clams and an estimate of mortality due to smashing or other harvesting-related damage which might have destroyed the notch mark. In each plot 15 cockles per 0.5 m<sup>2</sup> were hand planted among naturally occurring cockles (to minimize disturbance that would be caused by their removal) and allowed to acclimatize for 24 hours. The experimental plots were disturbed, after 24 hours, using three different harvesting techniques: spade, pitchfork and finger ploughing; there was also a control in which no disturbance was imposed. Experimental plots were harvested after three weeks and all marked cockles were removed (Robinson and Rowell

1990). Cockles were classified as dead if the shell was empty or had begun to gape and did not close when touched. Therefore, the harvesting process was simulated and no cockles were actually removed from the experimental plots.

#### Results

Figure 2A indicates that significant mortality occurred in the Moulting Lagoon plots which were disturbed using digging implements, i.e., spade or pitchfork. Both implements caused turnover of large tracts of sediment and subsequent burial of the test animals. No deaths were found due to harvesting damage (i.e., shell breakage). Harvesting using a spade caused an average of 43.4% mortality, while plots disturbed using a pitchfork caused 38.4% mortality of the experimental animals. The control treatment showed no mortality during the study period, and the finger ploughing technique caused the death of only one cockle.

A non-parametric Kruskal-Wallis analysis of variance (Table 1) showed significant differences in mortality evident between the harvesting techniques ( $p = 0.0001$ ). *A posteriori* testing identified two discrete groups of treatments: the finger ploughing and control plots with low levels of mortality and the spade and pitchfork plots with high levels of mortality.

Results from Pipe Clay Lagoon (Fig. 2B) showed the same pattern. The spade treatment caused 43.4% mortality on average, while the pitchfork treatment caused 31.7% mortality of the test animals. Finger ploughing resulted in the death of two cockles over the four plots and, again, the control showed no mortality. Mortality levels in each treatment did not vary significantly between Moulting Lagoon and Pipe Clay Lagoon (Table 1).

#### BURIAL EXPERIMENT

Results from the first part of the study suggested that substrate disturbance caused by some harvesting techniques causes mortality of experimental cockles. Implements which cause the turnover of large tracts of sediment, and the subsequent burial of cockles beneath the tailings, appear to cause increased mortality of *K. scalarina*.

It has been suggested that the primary problem for macrofauna faced with substrate disturbance is the loss of a favored position in the substrate (especially if the individual is infaunal) and also the loss of sediment stability (Jackson and James 1979, McLusky et al. 1983, Robinson and Rowell 1990). The mortality appeared to be due to the cockles being either buried so deeply in the sediment that they could not regain their normal subsurface position, or being exposed on the surface (Rice et al. 1989, Emerson et al. 1990, Robinson and Rowell 1990, Lush 1992).

The aims of this part of the study were: i) to determine whether

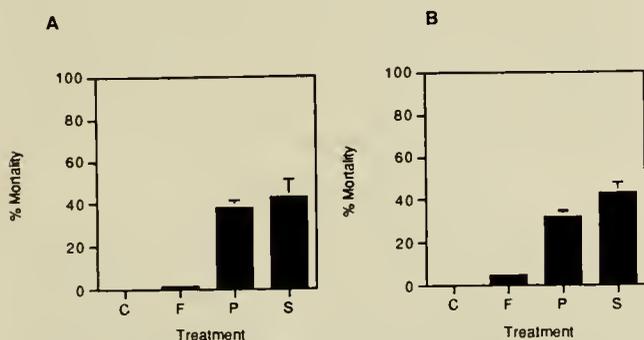


Figure 2. The effects of harvesting techniques on mortality of *K. scalarina* at (A) Moulting Lagoon and (B) Pipe Clay Lagoon. C: control, F: finger ploughing, P: pitchfork, S: spade. Vertical bars represent standard deviations.

TABLE 1.

Results from a 2-way Kruskal-Wallis analysis of variance of the effects of site and harvesting method on the mortality of *Katelysia scalarina*.

Source	SS	DF	H	P
A	0.031	1	0.001	0.9795
B	429.000	3	7.232	0.0001
AB	5.094	3	0.086	0.9667

A = Site (Moulting Lagoon, Pipe Clay Lagoon).

B = Harvesting method (Control, Spade, Pitchfork, Finger ploughing).

burial depth had a significant effect on the mortality of *K. scalarina*, ii) establish the critical burial depth, below which a low probability of recovery exists, and iii) to determine whether mortality rates differed in adults and juveniles following burial.

#### Methods

Burial treatments were conducted at both Moulting Lagoon and Pipe Clay Lagoon towards the low tide mark. Two size classes of cockles, adults (3–4.5 cm) and juveniles (<3 cm), were placed in natural positions in sand, inside acrylic cylinders (100 mm in diameter). Each cylinder contained four cockles marked with small notches filed into one valve. Sand was added to give burial treatments of different depths, chosen to reflect typical depths of burial during harvesting. Sand was sieved before being used in the treatments to remove potential predators. Five different burial depths were used (0 cm, 5 cm, 10 cm, 20 cm and 30 cm). Four replicates of each burial depth for both adults and juveniles were established at each of the two sites. Cockles were left for three weeks before reharvesting.

#### Results

A three-way Kruskal-Wallis ANOVA showed that site and burial depth (but not size) had significant effects on mortality (Table 2). Approximately 55% of cockles in the 20 cm, burial treatments, and 95% of cockles in 30 cm treatments died at Moulting Lagoon, but no mortality was observed at depths equal to, or less than, 10 cm (Fig. 3A, B).

Mortality rates were greater at Pipe Clay Lagoon over all the treatments and the controls (Fig. 3C, D). With greater than 90% mortality of both adults and juveniles in treatments greater than or equal to 20 cm.

There was a significant interaction between burial depth and size since adults were more susceptible to mortality following burial. Combined with the high mortality of juveniles at Pipe Clay this resulted in a significant interaction between all three treatment factors.

#### DISCUSSION

The results indicate that disturbance using a pitchfork or spade resulted in significantly greater mortality than finger ploughing at both sites. This is consistent with the results of Robinson and Rowell (1990) who found that increased mortality occurred in

TABLE 2.

Results from a 3-way Kruskal-Wallis analysis of variance of the effects of site, clam size and burial depth on the mortality of *Katelysia scalarina*.

Source	SS	DF	H	P
A	7277.11	1	15.79	0.0001
B	720.0	1	1.56	0.2085
C	17357.37	4	37.67	0.0000
AB	1487.81	1	3.23	0.0687
AC	781.58	4	1.70	0.1463
BC	2189.38	4	4.75	0.0009
ABC	2748.38	4	5.96	0.0001

A = Site (Pipe Clay, Moulting Lagoon).

B = Size (Adult, Juvenile).

C = Burial Depth (0, 5, 10, 20, 30 cm).

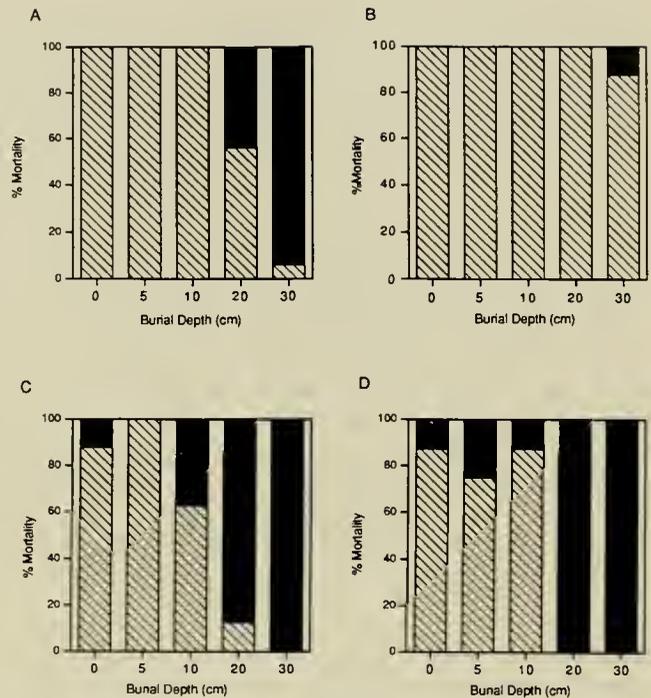


Figure 3. The effect of experimental burial treatments on the mortality of *Katelysia scalarina*. (A) adults at Moulting Lagoon, (B) juveniles at Moulting Lagoon, (C) adults at Pipe Clay Lagoon, (D) juveniles at Pipe Clay Lagoon. Solid bars represent % mortality, hatched bars represent % survival.

plots in Nova Scotia and New Brunswick which had experienced recent harvesting with clam hacks. They suggested that the increased mortality was due to increased predation pressure as sediment disturbance would undoubtedly have attracted predators (Robinson and Rowell 1990). This appears consistent with the results obtained in this study as there was no evidence (i.e., broken shells) that mortality was due to harvesting damage, and the majority of the marked cockles were recaptured as empty shells, suggesting that the animals were smothered or preyed upon. Although no predators were observed consuming the test animals (nor were there any signs of shell damage suggesting invertebrate predation), both of the study sites have large wading bird and invertebrate populations (crabs and carnivorous gastropods), making post-harvesting predation plausible.

The levels of mortality observed are consistent with those found in previous studies, (e.g., average mortality values of 30–70% for harvesting conducted using a spade. Emerson et al. 1990). However, Emerson et al. (1990) noted that harvesting using a pitchfork apparently causes lower mortality (30–60%). This may be because, compared to a spade, the pitchfork does not produce large clumps of sediment. It follows that any reduction in the size of tailing piles is likely to enhance the survival of both buried and exposed clams (Emerson et al. 1990).

The investigation of the effects of tailings on the survival of unharvested cockles showed that burial depth appeared to be a critical factor in causing mortality. Several authors have suggested that if harvesting causes an increase in the depth at which unharvested clams live, future population densities may be affected, because survival and growth of infaunal, suspension feeders are inherently linked to their distance from the sediment water interface (Quammen 1984, Emerson et al. 1990). Burial experiments

conducted on *Mya arenaria* indicate that clams were able to survive a 15 cm burial, but it appeared that any further increase in burial depth (25–75 cm) resulted in almost total mortality of all size classes (Emerson et al. 1990). Results from the present study are consistent with these findings as mortality was significantly increased when *K. scalarina* was buried at depths greater than 10 cm.

It is interesting that cockle mortality varied between the two sites, with greater mortality of adults and juveniles at Pipe Clay Lagoon. This may be related to differences in substrate, since Pipe Clay has an extensive shell grit layer only a few centimeters below the sand surface, while at Moulting Lagoon the shell grit layer was markedly reduced and deeper. However, as a detailed sediment analysis was not conducted, it can only be suggested that sediment characteristics may be responsible for the differences observed. It is not immediately obvious why the difference in substrate type should alter mortality rates although it has been suggested that differences in sediment type may enhance or inhibit the ability of the clams to reburrow to their normal subsurface position (Rice et al. 1989, Emmerson et al. 1990).

A significant difference in mortality was associated with the interaction between size and burial depth, with juveniles typically displaying lower mortality at a greater depth. The ability of juveniles to better withstand burial may be explained by their increased mobility through sediment (Rice et al. 1989). However, previous studies have shown that juvenile clams (*Mercenaria mercenaria*) generally display greater mortality than adults when exposed to burial treatments, due to their reduced ability to withstand smothering or suffocation (Rice et al. 1989, Emerson et al. 1990, Robinson and Rowell 1990). Differences in the sediment grain size, acclimation time and burial method may account for this deviation from other studies.

In conclusion, harvesting type and burial depth are significant factors in determining cockle mortality following the substrate disturbance associated with harvesting. The results of this study indicate that harvesting processes involving the use of digging implements to turn over large tracts of sediment have significant impact on the remaining population. This type of reworking would also have a high impact on the status of larvae or juveniles (Rice et al. 1989, Emerson et al. 1990, Lush 1992). The incidental digging mortality of cockles resulting from digging has potentially serious implications for the proper management of clam harvesting. This study indicates that finger ploughing is the least disruptive method of clam harvesting as it causes the least amount of burial of unharvested cockles. In terms of the long-term sustainability of *K. scalarina* populations this technique should be encouraged.

This work was primarily conducted as a pilot study which has stressed the need for further research if the clam industry in Tasmania is to survive and flourish. Based on the results obtained from this work and its correlation with previous studies, fisheries managers have moved to prohibit the mechanical harvesting of clams within the state until further research can be conducted. Due to the high efficiency of the finger ploughing method, the policies implemented are at this stage acceptable to fishermen and managers alike.

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## ASSESSMENT OF THE OCEAN QUAHOG, *ARCTICA ISLANDICA* (LINNAEUS, 1767), IN THE NEW JERSEY FISHERY

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**ABSTRACT** The status of the ocean quahog (*Arctica islandica*) resource off the New Jersey coast is described. Commercial landings and federal shellfish research survey data are analyzed to provide an update of the ocean quahog fishery in this area of the Mid-Atlantic region. Spatial and temporal trends in resource abundance, biomass, and size structure of the New Jersey ocean quahog fishery are compared to the data trends of other assessment regions along the East Coast. In addition, the population dynamics (i.e., reproduction, recruitment, growth, and mortality) of this species are examined to explain observations in the fishery. Ocean quahog populations off New Jersey consist of large (>70 mm in length), old (median age >70 years), and slow-growing individuals. Due to the absence of recruitment, very slow adult growth rates, low rates of adult mortality, and long time to maturity, there is little interannual variability in population size or structure. These unusual characteristics make the ocean quahog vulnerable to commercial exploitation. Hence, while New Jersey was the principal area of ocean quahog landings in the United States during the early 1990s (peak catch = 12,011 mt in 1990), concentrated fishing activity reduced the catch-per-unit effort substantially by 1992, and it now appears to have depleted the stock. As a result, the primary fishing grounds for ocean quahogs have recently shifted northward to less exploited areas.

### INTRODUCTION

The ocean quahog, *Arctica* (= *Cyprina*) *islandica* Linne, supports a multimillion dollar fishery off the Atlantic Coast of the United States. While a viable commercial fishery has existed for years from the Gulf of Maine to Cape Hatteras, the Mid-Atlantic Bight, particularly off the coast of New Jersey and Delaware, has provided the peak ocean quahog fishery during the past decade, owing principally to the proximity of land-based shucking facilities in this region. The concentrated fishing activity off New Jersey and Delaware has contributed to local stock depletions, and most recently, commercial landings data reflect a northward shift in the fishing effort.

The ocean quahog has become increasingly important to the sea clam industry in the U.S. since the mid-1970s, when an acute reduction in the landings of Atlantic surf clams caused by overfishing (Serchuk et al. 1979) prompted the shellfish industry to commence intense harvest of offshore clam beds (Jones 1981). As a result of this harvesting program, a major ocean quahog fishery developed in the Mid-Atlantic Bight, especially off New Jersey and the Delmarva Peninsula, which yielded substantial quantities of ocean quahog meats. In this region, commercial concentrations of the clam were consistently found at depths between 25 and 60 m.

From 1967 to 1975, U.S. landings of ocean quahogs were derived exclusively from state waters, with annual catches averaging less than 1000 mt of shucked meats (Table 1). Annual U.S. landings for this species increased from 569 mt of shucked meats to 15,748 mt between 1975 and 1979. This large increase in landings occurred subsequent to the inception of the offshore ocean quahog fishery in 1976. By 1978, most landings (>90%) were derived from the Exclusive Economic Zone (EEZ). Annual landings of ocean quahog meats averaged more than 16,100 mt of meats from 1979 to 1984. A record of 23,566 mt of meats was landed in 1985. Between 1979 and 1987, the ocean quahog fishery accounted for 45 to 50% of the sea clam meat landings; higher landings were registered during years when surf clam landings decreased. U.S. landings in 1989 equalled 23,145 mt of meats having an estimated dockside value exceeding \$16 million (U.S. Department of Commerce 1990). In 1991, 1992, and 1993, com-

mercial landings of *A. islandica* amounted to 22,287 mt of meats, 22,869 mt, and 21,838 mt, respectively, with most landings derived from the EEZ off New Jersey and the Delmarva Peninsula (Mid-Atlantic Fishery Management Council 1992, U.S. Department of Commerce 1993). Presently, annual landings represent about 2 to 6% of the total harvestable stocks.

Whereas the Delmarva Peninsula had the greatest landings and fishing effort between 1982 and 1989, New Jersey became the principal area for commercial exploitation of ocean quahogs during the early 1990s. However, concentrated fishing activity off New Jersey reduced the catch-per-unit effort (CPUE) by 29% between 1986 and 1992, and it now appears to be depleting the stock (Weinberg 1993). According to Weinberg (1993), the ocean quahog resource off New Jersey may be exhausted within 10 years at the current removal rates.

### OCEAN QUAHOG FISHERY

#### *Historical Development in New Jersey*

The ocean quahog commercial fishery in the U.S. initially developed off the coast of Rhode Island during the mid-1940s in response to the U.S. war food production program (Arcisz and Neville 1945). Between 1944 and 1976, annual landings of this species remained low (<1825 mt), with virtually all domestic production concentrated in the Rhode Island and Block Island Sound fisheries. The ocean quahog fishery did not develop in New Jersey until 1976 when steadily declining yields of Atlantic surf clam (*Spisula solidissima*) stocks, together with a massive natural kill of surf clams, caused a shift in fishing effort to the ocean quahog in deeper offshore waters (Mid-Atlantic Fishery Management Council 1992). Owing to extensive beds of ocean quahogs and the proximity of land-based shucking facilities, the New Jersey and Delaware fisheries accounted for the majority of landings from the Mid-Atlantic region during the 1980s.

In 1990, New Jersey became the focus of the ocean quahog fishery in the United States, yielding a total catch of 12,011 mt (Table 2). A similar harvest (12,002 mt) was realized in 1991; most of these landings were derived from the EEZ. With an estimated dockside value of more than \$7 million, ocean quahog

TABLE 1.

Annual landings of the ocean quahog (metric tons, meats) from state waters and the Exclusive Economic Zone.<sup>1</sup>

Year	State Waters	EEZ	Total	% EEZ
1967	20	—	20	0
1968	102	—	102	0
1969	290	—	290	0
1970	792	—	792	0
1971	921	—	921	0
1972	634	—	634	0
1973	661	—	661	0
1974	365	—	365	0
1975	569	—	569	0
1976	656	1854	2510	74
1977	1118	7293	8411	87
1978	1218	9197	10,415	88
1979	1404	14,344	15,748	91
1980	1458	13,885	15,343	90
1981	410	15,966	16,375	97
1982	207	15,572	15,779	99
1983	701	15,228	15,978	96
1984	1200	16,401	17,602	93
1985	—	23,566	23,566	99 <sup>2</sup>
1986	814	19,771	20,585	96
1987	569	22,226	22,795	98
1988	412	20,594	21,006	98
1989	184	22,996	23,145	99
1990	116	21,079	21,195	99
1991	40	22,246	22,287	100
1992 <sup>3</sup>	—	22,461	—	—

<sup>1</sup> Landings through 1991 are based on data in U.S. Department of Commerce 1992 and in Murawski et al. 1990.

<sup>2</sup> Some inshore landings were from Maine coastal waters. However, the magnitude of the fishery was small, and catch statistics are not available.

<sup>3</sup> The 1992 landings were estimated from data available on October 20, 1992. Landings for 1992 came from the Mid-Atlantic Bight (55%), Georges Bank (0%), Southern New England (45%), and the Gulf of Maine (0%).

Data from Weinberg, J. R., 1993.

catches during the early 1990s constituted about 40% of the national landings and dollar value of this species. These landing statistics are far in excess of those of other shellfish species with the exception of the Atlantic surf clam, which comprises approximately 45% of the total annual sea clam production in New Jersey.

Fishing pressure appears to have affected the resource in both the New Jersey and Delmarva fisheries, as reflected by declining CPUE values since the mid-1980s as noted previously (Table 2). Annual commercial vessel CPUE values have clearly shown significant declines in heavily fished locations within these two areas (Mid-Atlantic Fishery Management Council 1992), although the CPUE in New Jersey has been greater than in Delmarva since 1990 (Weinberg 1993). In addition, there has been a perceptible decrease in CPUE in the southern range of the resource, notably off Maryland and Virginia.

#### Stock Assessment

Federal exploratory and shellfish research surveys have been conducted by the National Marine Fisheries Service (NMFS) and its predecessor, the Bureau of Commercial Fisheries, since 1965 to

obtain information on the temporal and spatial distribution, size structure, and relative abundance of ocean quahog populations in the Mid-Atlantic Bight and other assessment regions (Murawski and Serchuk 1979, 1989, Ropes 1979, Fogarty 1981, Murawski et al. 1990, Weinberg 1993). These surveys were conducted most frequently between 1965 and 1985. Only three surveys (1986, 1989, and 1992) were completed after 1985. While federal research surveys have provided an independent measure of temporal changes in population size and structure in the assessment areas, commercial landings contained in vessel logbooks obtained from all participants in the fishery have yielded estimates of the fraction of ocean quahog stocks that has been removed from shellfish beds. The Mid-Atlantic Fishery Management Council has utilized these data in its Ocean Quahog Fishery Management Plan to regulate catch quotas in the fishery. During the past decade, the annual harvest quota for ocean quahogs has amounted to only about 2% of the overall standing stock (Weinberg, 1993). In 1992, for example, the ocean quahog quota for the EEZ was 5,300,000 bu (24,400 mt of shucked meats) (Mid-Atlantic Fishery Management Council 1992).

#### NMFS Research Surveys

Prior to 1978, federal surveys of offshore ocean quahog resources were based on a grid-type sampling design, with stations located along either LORAN or latitude-longitude lines at approximately 16 km intervals. Subsequent surveys employed a stratified-random design in which strata selection was based primarily on depth and, to a lesser extent, on bottom type (Fig. 1). Strata were assigned to geographical assessment areas (i.e., Gulf of Maine, Georges Bank, Southern New England, Long Island, New Jersey, Delmarva, and Southern Virginia — North Carolina), and a predetermined number of tows was allocated to each stratum. Survey gear consisted of a commercial-type hydraulic clam dredge (1.5 m wide) modified to retain small clams. At each station, the

TABLE 2.

Summary of the annual ocean quahog catch (metric tons), effort (thousands of hours fished) and catch-per-unit effort (CPUE, kilograms per hour fished) for the New Jersey fishery.<sup>1</sup>

Year	Sum <sup>2</sup>	Catch <sup>3</sup>	Effort	CPUE
1979	9680	6772	11.6	585
1980	7570	6332	13.0	485
1981	5869	5166	9.3	553
1982	2118	1728	3.5	499
1983	3960	3493	6.4	549
1984	4699	3887	7.2	535
1985	6994	6164	10.3	599
1986	7321	6740	10.1	662
1987	7480	6840	10.4	658
1988	4704	4577	7.3	630
1989	8723	7965	14.0	567
1990	12,011	10,351	18.8	549
1991	12,002	10,392	21.7	476
1992 <sup>4</sup>	6201	4894	10.4	467

<sup>1</sup> Loran C coordinates 42650–43700.

<sup>2</sup> "Sum" is sum of all landings by all vessel classes.

<sup>3</sup> "Catch" is catch by Class 3 vessels used in the CPUE index.

<sup>4</sup> Estimated.

Data from Weinberg, J. R., 1993.

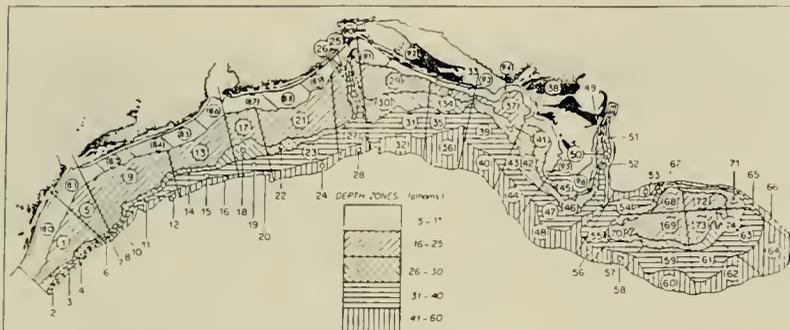


Figure 1. Sampling strata used for NMFS hydraulic clam dredge surveys in assessment areas off the northeast U.S. From Murawski and Serchuk, 1989.

survey dredge was towed for 5 minutes duration, thereby enabling the survey catches to be later enumerated and standardized. By applying appropriate length-weight equations to numbers caught in each 10 mm size category (Murawski and Serchuk 1979), the catch was computed in total meat weight per tow. Murawski and Serchuk (1989) provide further details of the standardized sampling procedure.

Figure 2 shows the size-frequency distribution of ocean quahogs per tow in the New Jersey assessment area for each survey period since 1980, and Table 3 lists the mean numbers and weights of clams per survey tow for these periods. As evident from Figure 2, the New Jersey assessment area is dominated by larger individuals greater than 70 mm in length, reflecting in part the failure of recruitment in the fishery over a considerable time interval. Analysis of the trends in the survey data indicates a significant decline in abundance indices. For example, during the 1980 to 1992 sam-

pling period, the stratified mean number per tow decreased from 99.16 to 68.06 individuals, which represents a reduction in density of 31.4%. The mean weight per tow decreased by 41% (4.08 to 2.39 kg/tow) between 1986 and 1992. When the 1992 abundance indices were compared among all survey regions, the New Jersey assessment area ranked near the bottom, with a mean number per tow of 68.06 (Fig. 3). The Southern New England, Long Island, and Georges Bank areas exhibited the highest abundance indices, amounting to 270.53, 263.89, and 207.55, respectively. The low abundance indices in the New Jersey assessment region in 1992 were accompanied by low biomass estimates relative to the other survey regions (Fig. 4). The biomass values in Figure 4 represent minimum swept-area biomass estimates for each area plotted on a percentage basis. Despite its low abundance and biomass estimates, the New Jersey assessment area had the most intense commercial fishery in 1992.

#### Commercial Landings Data

Although research vessel surveys in the New Jersey assessment area did not reveal significant population reductions in exploited beds during the 1980s, the evaluation of commercial vessel performance has indicated significant decreases for some areas. For example, of the eight ten-minute squares off southern New Jersey where more than 1 million bu of clams have been removed, the CPUE dropped from an average of 140 bu/hr in 1983 to 86 bu/hr in 1991. Even more dramatic has been the reduction of CPUE in areas where more than 1 million bu of quahogs have been removed off Delmarva. Here, the CPUE averaged 180 bu/hr in 1983, but only 80 bu/hr in 1991 (Mid-Atlantic Fishery Management Council 1992).

Table 2 presents a summary of the annual commercial landings and fishing effort off New Jersey from 1979 to 1992. Landings by all vessel classes ranged from a low of 2118 mt in 1982 to a high of 12,011 mt in 1990. After 1982, the landings increased gradually to 7480 mt in 1987, but declined to 4704 mt in 1988. However, they increased once again to 12,002 mt in 1991. The catch decreased markedly to an estimated 6201 mt in 1992, along with the fishing effort which decreased sharply from 21,700 hours fished in 1991 to 10,400 hours fished in 1992. Of the 13 ten-minute squares from all assessment areas within which fishermen reported an annual catch of more than 1 million bu during the 1983-1991 period, eight are off southern New Jersey. These 13 squares, together with four others within which more than 500,000 bu have been removed, yielded approximately 75% of the total EEZ landings during this nine-year period. These landings statistics dem-

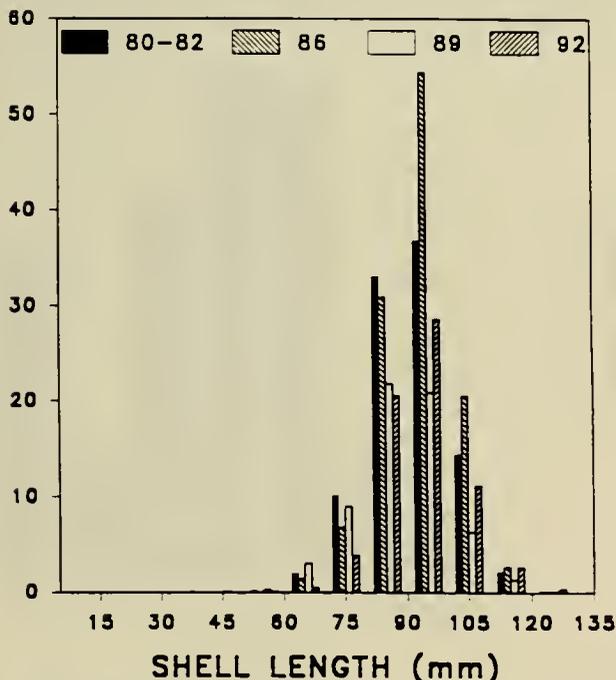


Figure 2. Length measurements of ocean quahogs from the New Jersey assessment area based on NMFS surveys conducted during four time periods: 1980-1982, 1986, 1989, and 1992. Data are expressed as the stratified mean number per tow taken in each 1 cm size interval. From Weinberg, 1993.

TABLE 3.

Research vessel survey indices for the ocean quahog in the New Jersey assessment area, 1980–1992, in stratified mean numbers and weights (meats, kilograms) per survey tow, standardized for five minutes.

Length <sup>1</sup>	Year of Survey Data				
	1980–1982 <sup>2</sup>	1986	1989	1992	All Data
10–19					
20–29			0.08		0.01
30–39	0.01	0.02	0.02	0.04	0.02
40–49	0.06	0.02	0.10	0.03	0.06
50–59	0.23	0.01	0.35	0.13	0.20
60–69	1.99	1.42	3.05	0.53	1.83
70–79	10.17	6.83	9.01	3.89	8.46
80–89	33.08	30.90	21.79	20.52	28.45
90–99	36.80	54.41	20.85	28.61	34.80
100–109	14.42	20.55	6.30	11.17	13.19
110–119	2.19	2.70	1.34	2.68	2.09
120–129	0.19	0.16	0.16	0.41	0.20
130–139	0.01	0.02		0.04	0.01
Stratified					
Mean	99.16	117.05	63.06	68.06	89.34
SD <sup>3</sup>	11.81	26.48	14.24	14.15	7.74
CV	0.12	0.23	0.23	0.21	0.09
n	360	105	111	104	680
Area of					
Surveyed					
Strata	7601	6856	7332	6856	.601
Mean					
Weight <sup>4</sup>					
Per Tow	3.246	4.084	1.921	2.388	2.957

<sup>1</sup> Length measurements in millimeters.

<sup>2</sup> Four surveys, two in 1980, one in 1981, one in 1992.

<sup>3</sup> SD is the standard deviation of the stratified mean per tow.

<sup>4</sup> Weight measurements in kilograms.

Data from Weinberg, J. R., 1993.

onstrate the overriding importance of the New Jersey resource to the entire East Coast commercial fishery during the 1980s and early 1990s.

#### POPULATION DYNAMICS

In 1977, the Mid-Atlantic Regional Fisheries Council implemented a management plan for the ocean quahog which included research initiatives on the biology and ecology of the species (Ropes 1979). Since the late 1970s, investigations on the population dynamics of *A. islandica* have accelerated, due to the increased importance of this bivalve to the sea clam industry. These investigations have generated much information on the reproduction, growth, and age structure of this valuable sea clam (Serchuk et al. 1970, Fogarty 1981, Jones 1981, Mann 1982, Murawski et al. 1982, 1989, Ropes 1984, Ropes et al. 1984a, Rowell et al. 1990, Fritz 1991, Kraus et al. 1991, Kennish et al. 1994). However, considerable uncertainty remains with regard to recruitment and mortality of *A. islandica*. In particular, questions have yet to be answered on the causes of the persistently poor recruitment off New Jersey and throughout the Mid-Atlantic Bight. These deficiencies have hindered accurate assessment of the maximum sustainable yield of this population.

#### Reproduction

*A. islandica* is dioecious; hermaphroditism occurs only rarely. Mann (1982) found morphologically ripe individuals from May through September in a population of clams in the vicinity of Block Island, RI. He also recorded a prolonged spawning period in this population from May through November, although spawning was most intense from August through November. Jones (1981), investigating the annual gametogenic cycle of *A. islandica* off New Jersey (predominantly offshore Asbury Park at 40°15'N, 73°40'W and secondarily offshore Cape May at 38°55'N, 74°25'W), stated that spawning appeared to be a fall- to early-winter event rather than summer- to early-fall as reported elsewhere. Spawning activity was greatest in the fall, but often persisted into the winter months. While some workers (i.e., Thompson et al. 1980) observed that *A. islandica* spawns at least once per annual reproductive cycle, Mann (1982) documented multiple annual spawning events at both the individual and population level. Temperature seems to be an important spawning stimulus (Loosanoff 1953, Jones 1981); however, it may be effective only in conjunction with changes in other stimuli, such as increases in percentage saturation of oxygen, pH, and food availability (Mann 1982).

The rates of gametogenic development may be highly variable within a population of ocean quahogs (Mann 1982, Rowell et al. 1990, Fritz 1991). For example, specimens collected by Mann (1982) in June on the Southern New England shelf were in all phases of gametogenic development, from early active through spent. Samples collected by Rowell et al. (1990) in April and May at a site off Nova Scotia, Canada, were in four of five phases of development.

During a spawning event, millions of eggs are released by females, and fertilization occurs in the water column. Viable lar-

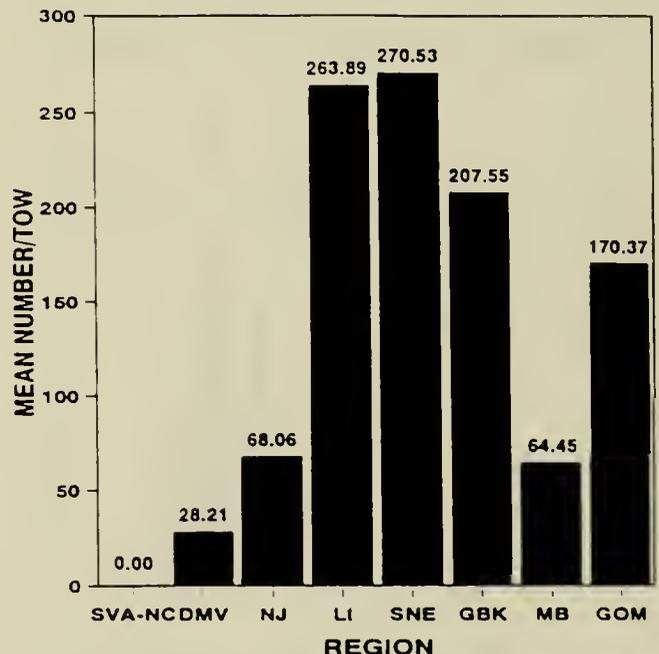


Figure 3. Abundance of ocean quahogs for assessment areas based on data from the 1992 NMFS research vessel survey. SVA-NC = Southern Virginia-North Carolina; DMV = Delmarva; NJ = New Jersey; LI = Long Island; SNE = Southern New England; GBK = Georges Bank; MB = Massachusetts Bay; GOM = Gulf of Maine. From Weinberg, 1993.

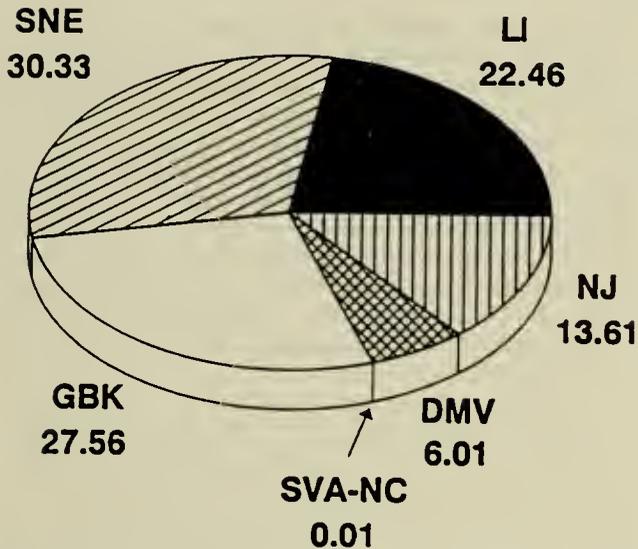


Figure 4. Ocean quahog biomass estimates for assessment areas based on NMFS research vessel surveys, 1986–1992. SVA-NC = Southern Virginia-North Carolina; DMV = Delmarva; NJ = New Jersey; LI = Long Island; SNE = Southern New England; GBK = Georges Bank. From Weinberg, 1993.

vae that are produced undergo typical molluscan development from trochophore through veliger stages to metamorphosis (Landers 1976). Larvae of *A. islandica* have been successfully reared to metamorphosis in the laboratory at temperatures between 9° and 13°C (Landers 1976, Lutz et al. 1982). The duration of larval life can be protracted, leading to a substantial wastage of numbers ascribable to predation and to the vagaries of environmental conditions. In laboratory culture at 10° to 12°C, a 60-day larval period has been determined for *A. islandica* (Lutz et al. 1982). The longevity of larval life in nature is largely dependent on food availability and the success of planktotrophic larvae to satisfy nutritional requirements.

#### Recruitment and Mortality

Recruitment of *A. islandica* from the plankton into soft-bottom habitats may be considered to be a two-phase process involving settlement of larvae followed by post-larval survival and growth to a size capable of retention on a 0.5 mm sieve (Feller et al. 1992). The variation in recruitment of benthic marine species with planktonic larvae, such as *A. islandica*, can be considerable (Caffey 1985, Connell 1985, Watzin 1986, Bertness et al. 1992, Feller et al. 1992, Gaines and Bertness 1992, Hurlbut 1992). However, it is unresolved at present what proportion of this variation is attributable to larval supply or early settler mortality. The relationship between larval supply and early settler mortality is often confounded because recruitment is commonly measured by taking a census of juveniles long after they have settled out of the water column (Keough and Downes 1982, Underwood and Denley 1984, Connell 1985, Bertness et al. 1992). As a result, understanding of the effects of variation in larval supply and early settler mortality of *A. islandica*, as well as many other benthic marine invertebrates, over space or time is largely inferential (Connell 1985, Gaines et al. 1985, Bertness et al. 1992).

The effects of environmental factors on the survival of juvenile stages of ocean quahogs remain open to discussion. Sediment type affects the distribution and density of ocean quahogs. In Rhode Island Sound and off Martha's Vineyard, MA, Fogarty (1981)

discerned the highest density of ocean quahogs in sediments consisting of high proportions of medium (0.25–0.49 mm) sand and shell fragments. The lowest density of the clams was delineated in sediments containing a high silt/clay fraction or coarse sand-gravel. These patterns suggest that ocean quahog larvae may preferentially settle on substrates composed of medium sand and shell fragments. Alternatively, survival of post-set clams may be greater in this type of sediment.

Temperature clearly influences the distribution of post-set clams. Juveniles and adults rarely exist along the seafloor where bottom water temperatures exceed 15.6°C (Mid-Atlantic Fishery Management Council, 1992). Predation may also contribute to high levels of mortality among post-set clams. The small size of these young clams and their inability to burrow deeply in sediments make them particularly vulnerable to predatory attacks (Ropes 1979). The combined effect of adverse environmental factors and predation on planktonic and post-set stages probably is responsible for the lack of recruitment observed in the Mid-Atlantic fishery during the last 30 years.

Mortality rates decline substantially after the first year of life and appear to be very low among larger, older clams. When individuals exceed approximately 20–30 mm in length and burrow more deeply in seafloor sediments, predation drops significantly. Overall adult mortality is low. Using 1981–1992 NMFS research vessel survey data, Weinberg (1993) calculated an instantaneous rate of mortality (Z) for ocean quahogs in the New Jersey assessment area of only 0.04. This mortality rate is consistent with a population in which a substantial proportion of individuals survive to more than 100 years (Murawski and Serchuk 1983, 1989).

#### Growth and Longevity

Data acquisition on growth rates and longevity of ocean quahogs is needed not only to establish reliable catch quotas, but also to evaluate the potential impacts of various harvesting strategies and other anthropogenic impacts on the resource (Murawski and Serchuk 1979, Murawski et al. 1982). Proper management of sea clam stocks is clearly dependent on accurate age and growth rate data, as well as reliable recruitment estimates of natural populations. During the last two decades, a number of studies have shown that the ocean quahog is an extremely slow-growing and long-lived species (Jones 1980a,b, Thompson et al. 1980a,b, Forster 1981, Ropes et al. 1981, 1984a,b, Murawski et al. 1982, Ropes and Pyoas 1982, Turekian et al. 1982, Ropes and Murawski 1983, Ropes 1984, Murawski and Serchuk 1989). Several techniques have been employed to validate growth and aging studies, including mark-recapture experiments, length-frequency analysis, and variability in external and internal shell banding patterns (Murawski et al. 1982, Ropes 1984, Ropes et al. 1984b, Murawski and Serchuk 1989). Based on shell banding studies, Ropes and Murawski (1983) reported age estimates as high as 157 years for ocean quahogs dredged off central New Jersey. Murawski and Serchuk (1983) projected that 17% of the New Jersey resource is greater than 100 years' old, with the median age of this exploited stock being greater than 70 years. These sea clams may take 20 years or more to reach commercial size, and depleted stocks can require 50 to 100 years to replenish themselves (Weinberg, 1993). These data, if correct, have serious implications for prudent management of this valuable resource.

A team of Rutgers University scientists investigated *in situ* growth rates of ocean quahogs in the New York Bight between

1984 and 1992. Kennish et al. (1994) assessed the growth of laboratory-spawned and Gulf of Maine ocean quahogs transplanted at a site of commercially important shellfish beds 65 km off of Cape May, NJ (39°00'45"N, 74°04'32"W) at a depth of 45 m. They found that the mean increase in shell length of adult clams from the Gulf of Maine amounted to .35 mm, .39 mm, -.02 mm, .40 mm, and -.10 mm when measured 213, 307, 368, 520, and 606 days after transplantation, respectively. The negative values may be attributed to shell dissolution during periods of anaerobic respiration (Lutz and Rhoads 1977).

Younger, laboratory-spawned clams exhibited greater growth rates than adults at the experimental site off Cape May. Their mean increase in shell length equalled 1.87 mm, 1.34 mm, 5.28 mm, .54 mm, and .16 mm when measured 75, 120, 288, 374, and 485 to 577 days after transplantation, respectively (Fig. 5). Kennish et al. (1994) concluded that smaller clams (<25 mm in length) appear to grow at rates of 10 to 12 mm per year, whereas larger clams (>50 mm in length) have substantially reduced growth rates and may not grow at all for long periods of time. These findings support those of earlier investigators (e.g., Jones 1980b, Thompson et al. 1980a, Murawski et al. 1980, 1982, Ropes et al. 1984a) who documented extremely slow growth rates of adult populations.

Murawski et al. (1982) recorded *in situ* growth rates of ocean quahogs planted at an experimental site in the New York Bight, located 48 km SSE of Shinnecock Inlet, Long Island, N.Y. (40°25.1'N, 72°23.7'W). By examining internal and external annuli formed in the shells of recovered specimens, Murawski et al. (1982) calculated the following increases in shell length of the experimental animals: 6.3% at age 10, 0.5% at age 50, and 0.2% at an estimated age of 100 years. They also formulated a growth rate relationship for clams 59–104 mm in shell length recaptured one year after their release ( $SL_{t+1} = 2.0811 + 0.9802 SL_t$ ), and an age/growth relationship [ $SL = 75.68 - 81.31 (0.9056)^t$ ] for younger, smaller individuals sampled from a natural population of unmarked clams in vicinity of the experimental site. On the basis of this work, ocean quahogs were shown to rank among the slowest growing bivalves inhabiting continental-shelf waters.

## DISCUSSION

The ocean quahog is a species vulnerable to commercial exploitation. Although ocean quahog stocks have remained relatively

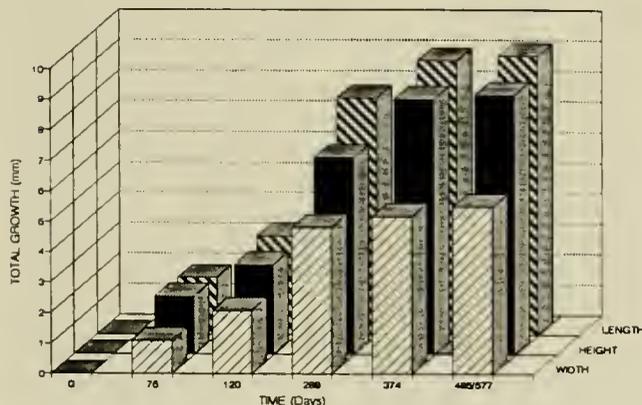


Figure 5. Mean cumulative growth rate (length, height, width) of laboratory-spawned ocean quahogs planted at the site of commercially important shellfish beds off Cape May, New Jersey and measured at intervals of 75, 120, 288, and 374 days. From Kennish et al., 1994.

stable since federal research survey assessment cruises were initiated in 1965, the characteristics of this species—rare and unpredictable recruitment events, slow growth rates, great longevity—have profound effects on fishery management policies. It is of utmost importance to establish reliable annual harvest quotas that ensure long-term viability of the resource. Protection of the fishery requires constant surveying of heavily exploited areas to delineate the response of the populations to intense harvesting pressure. This program is consistent with the objectives of the initial fishery management plan of 1977 designed to prevent the harvest of ocean quahogs from exceeding biologically sound levels, and to direct the fishery toward maintaining optimum yield (Mid-Atlantic Fishery Council 1977).

To determine optimum exploitation rates for maximizing the yield potential of ocean quahog cohorts, the NMFS performed a series of yield-per-recruit (Y/R) analyses (Murawski et al. 1982). These calculations indicated that exploitation rates greater than 2–5%/yr would result in overfishing of the stock. However, the Y/R analyses assumed constant annual recruitment of the stock, and as research survey data have indicated, recruitment has been extremely poor in the fishery during the past three decades. Hence, optimal exploitation rates as determined by Y/R analyses do not result in stable ocean quahog stock sizes (Murawski and Serchuk 1989).

Murawski and Serchuk (1983) and Murawski et al. (1982) suggested that the extremely slow growth rates and poor recruitment of this species signify a resource at the carrying capacity of the ecosystem. Murawski and Serchuk (1989) inferred that areas subjected to intense fishing pressure, such as offshore New Jersey, should exhibit increased growth and recruitment rates in the fishery as clam density is reduced, if density-dependent population regulatory mechanisms are contributing to the low productivity of the ocean quahog stock. However, heavily fished areas (e.g., New Jersey and Delmarva) have not exhibited new recruitment or accelerated growth rates in response to declining densities. New Jersey presently ranks fourth among six geographical assessment areas in terms of total density of quahog beds. The rankings are as follows:

1. Southern New England
2. Long Island
3. Georges Bank
4. New Jersey
5. Delmarva
6. Southern Virginia—North Carolina

Due to the absence of recruitment, long generation time, slow adult growth rates, and low rates of adult mortality, there is little interannual variability in ocean quahog population size or structure off New Jersey and throughout the Mid-Atlantic region (Weinberg 1993). However, intense fishing appears to significantly impact population structure on a local basis. This is evident in heavily harvested areas off New Jersey and the Delmarva Peninsula, where the removal of more than a million bushels of quahogs has caused local stock depletions. These heavily exploited areas exhibited a marked decline in CPUE through the 1980s and early 1990s. Approximately 31% and 45% of the resource off New Jersey and Delmarva, respectively, had been harvested by 1993 (Weinberg 1993). The New Jersey fishery, consisting of large individuals (>70 mm in length) with a median age exceeding 70 years, would be exhausted within a decade or two at removal rates of the early 1990s. Once depleted, such heavily harvested ocean quahog beds could require 50 to 100 years to replenish themselves (Weinberg 1993). This is so because ocean quahogs require more

than 20 years to grow to commercial size, and good recruitment events are rare. In response to the diminishing resource performance off New Jersey, the fishery has gradually moved north to less exploited areas.

One of the primary goals of the Mid-Atlantic Fishery Management Council is to protect the viability of the ocean quahog fishery through the establishment of reliable annual catch quotas. The council considers current stock assessments, prior catch statistics, population structure, and other relevant information when it formulates catch quotas for the fishery. The annual EEZ ocean quahog quotas recommended by the council for the fishery during the 1980s were between 4 and 6 million bu. From 1990 through 1993, the quotas were the same, 5.3 million bu. The quota increased slightly to 5.4 million bu in 1993. These quotas are deemed to be conservative values that not only protect the resource, but also provide for growth at a limited rate (Mid-Atlantic Fishery Management Council 1992). Logbook reports indicate, for example, that only 87% and 91% of the 1990 and 1991 quotas, respectively, were actually landed. The council has been careful over the years not to greatly increase or decrease the quotas, thereby mollifying any economic disruption to the commercial fishing industry.

The success of management programs will be closely coupled to studies of the population dynamics of this species. Future research should be conducted on the dynamics of the early life history stages of the ocean quahog in nature. Investigations should also be undertaken on recruitment in the species and the factors that may be responsible for the persistent lack of recruitment success in the Mid-Atlantic region. Proper management of the ocean quahog resource is clearly dependent on accurate standing stock estimates, age and growth rate data, recruitment, and mortality. While there is a gradually accumulating database on the growth rates and age structure of ocean quahogs along the Mid-Atlantic

Bight, including New Jersey, there is a dearth of information on post-set survivorship and recruitment of clams in this region. Data on early juvenile survival and recruitment of ocean quahogs in exploited populations off New Jersey are critically important to decision makers in the sea clam industry and government fisheries programs who must provide effective long-term management of this multimillion dollar resource. The paucity of survivorship and recruitment data, in particular, places a constraint on the establishment of precise optimum exploitation rates for maximizing the yield potential of the species.

Equally important is the need to conduct research on the anthropogenic impacts on commercially important shellfish beds. In particular, investigations should focus on the effects of hydraulic dredging on both sensitive habitat areas and the clam populations themselves. Only through careful analysis of the natural and anthropogenic factors affecting ocean quahogs can effective long-term management of the resource be achieved.

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## CHANGES IN SOMATIC AND REPRODUCTIVE TISSUES DURING ARTIFICIAL CONDITIONING OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791)

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**ABSTRACT** The changes in adductor muscle and gonad tissue weights, and corresponding changes in RNA/DNA ratios, were analyzed during artificial conditioning of the sea scallop (*Placopecten magellanicus*) at two locations. The scallops took approximately 95 days to condition and spawn from a spent state. In contrast to wild populations, the adductor muscle weight did not decline significantly during gametogenesis. There was a strong negative correlation between gonad and muscle RNA/DNA throughout conditioning. RNA/DNA ratios in the muscle peaked prior to spawning, whereas the gonad RNA/DNA was at a minimum at this time. Throughout, the mean weight of the adductor muscle was more than twice that observed in the wild during gametogenesis, indicating that the scallops were accumulating reserves despite the stress of gamete production. As food production is a costly and often limiting aspect of hatchery operations, it may prove beneficial to manipulate the use of endogenous reserves to produce conditioned broodstock with less food.

**KEY WORDS:** Conditioning, RNA/DNA, adductor muscle, gonad, sea scallop, *Placopecten*

### INTRODUCTION

The Atlantic sea scallop, *Placopecten magellanicus* (Gmelin 1791), is the most commercially important bivalve in North America (Naidu 1991). The greatest portion of the revenue produced from this species is procured from the wild fishery. However, the sea scallop has a number of qualities rendering it amenable to aquaculture (Naidu et al. 1989). Presently there is a small scallop aquaculture industry in Atlantic Canada. With one exception (see below), this industry relies on the wild harvest of spat to supply growers. Recently, Canadian hatcheries and research institutes have experimented with the artificial production of native bivalve species including *P. magellanicus*. Hatchery production of *Placopecten* has the potential to supply growers with a more consistent source of spat. Artificial manipulation of gametogenesis from a typical autumnal spawn to a spring spawn may also reduce significantly the grow-out time for this species (Dadswell and Parsons 1992).

To prompt a spring spawn, scallops must be induced to undergo gametogenesis earlier than would occur in most localities in the natural environment. This process is referred to as "conditioning" the animal. Conditioning of bivalves is achieved by manipulation of two factors: temperature and food (Loosanoff and Davis 1963). While sea scallops have been successfully produced through conditioning and spawning, the process has not been critically examined to optimize production at minimal cost. Elucidating aspects of broodstock biology during artificial conditioning will provide insight into this process.

Like many other bivalves, scallops have seasonal cycles of energy utilization which are intimately associated with the reproductive cycle (Ansell 1974, Robinson et al. 1981, Barber and Blake 1991, Besnard 1991). A quality common to all cycles is the utilization of energy reserves stored in various body components. Variation of body component weights indicates either accumulation or utilization of storage substrates (Barber and Blake 1991). In

a two-year study of wild populations of inshore Bay of Fundy scallops, Kenchington et al. (1994) monitored seasonal variations in gonad and adductor muscle weight. Gonad weight increase (April–September) coincided with a decrease in adductor muscle weight. Adductor weight decrease during gametogenesis, and adductor recovery following the spawn, suggest the transfer of substrates from the adductor during gametogenesis. Another possibility is that resources are directed preferentially toward gamete production at the expense of the muscle. A study monitoring the weight variation of sea scallop adductor and gonad tissue has not yet been conducted during broodstock conditioning. During artificial conditioning, it is possible that the biochemical cycling observed in wild gametogenesis may be less dependent on endogenous reserves, and may shift to a greater utilization of nutrients from exogenous food, which in wild stocks is only seasonally available in abundance. This study presents an analysis of the changes in somatic and reproductive tissue weights during artificial conditioning.

Metabolic activity in these tissues was also examined through changes in RNA/DNA ratios which have been shown to be a sensitive, reliable biochemical index that can serve as an indicator of condition (cf. Kenchington 1994). The amount of DNA remains relatively constant within the somatic cells of a species (Clemmesen 1993). Whereas, RNA content is known to vary with age, life-stage, organism size, disease-state and with changing environmental conditions of food availability, temperature and pollution (Pease 1976, Bulow 1987). RNA is an obligate precursor in protein biosynthesis (Robbins et al. 1990), thus, the ratio provides a self-calibrating index of protein synthesis within a species.

RNA/DNA ratios have been previously used for determining condition and growth in scallop species. In the Chilean scallop, *Argopecten purpuratus*, RNA/DNA ratios of gonad tissue were studied to compare hatchery-reared scallops with ocean-reared stock (Martinez et al. 1992). During a year-long study, Robbins et al. (1990) monitored the RNA/DNA in the gonad tissue of *Pecten*

*maximus* and observed seasonal variation related to synthetic activity and reproductive state. For *Placopecten*, the ratio has been monitored over a three-year period, in wild stocks (Kenchington 1994), however, RNA/DNA has not been investigated during the artificial conditioning of this species.

### MATERIALS AND METHODS

Sea scallops were collected from a commercial grow-out site at Whitehead, N.S., on November 4, 1993, and held in ambient seawater at the Fisheries Resource and Development Ltd. (FRDL) hatchery. The scallops were all from the same hatchery spawned cohort, which originated from Passamaquoddy Bay, New Brunswick. On November 10, 120 scallops were transferred to the aquarium at the Department of Fisheries and Oceans (DFO), Halifax, N.S., for conditioning coincident with the initiation of conditioning in the FRDL broodstock tanks. Scallops were simultaneously conditioned at both sites. At the time of collection, scallops demonstrated both partially and fully spent (post-spawn) reproductive states. When the DFO scallops were fully conditioned, sperm and ova were collected and placed in a bucket of seawater at 15°C to allow fertilization. Larval development was monitored by microscopic examination to confirm that viable gametes were produced through the conditioning process.

Sea scallops were conditioned at the FRDL hatchery according to hatchery protocol. The details of the conditioning were not made available to us, but are presumably modifications of controlled temperature and feeding regimes.

The sea scallops at DFO were placed in a 1000L flow-through tank, to which filtered seawater was constantly supplied at 61 min<sup>-1</sup>. The animals were fed a mixture of *Isochrysis galbana* (Tahitian strain), and *Chaetoceros muelleri* (syn. *C. gracilis*) algae (Grant and Cranford 1989). A mean temperature of 7°C was maintained during conditioning. Photoperiod was 8:16 hours light to dark.

Scallop body component weights were monitored every 2 weeks ( $n = 10$ ) in the DFO lab stock until January 20. At this point macroscopic observations suggested a transitional phase in gonad development. After January 20, weekly samplings ( $n = 5$ ) were taken until spawning. At the FRDL site, 5 animals were randomly chosen from the hatchery broodstock at 2-week intervals for the first month, then monthly for the remainder of the experiment. This sampling size and frequency was limited by the constraints of FRDL hatchery facilities.

The wet weights of the adductor muscle and gonad were recorded at each sampling to the nearest 0.01 g using a Mettler BB120 digital balance. Adductor muscle and gonads were then packaged, cross-labeled, and stored in a freezer at -85°C for later analysis of RNA/DNA ratios. Shell heights were measured to the nearest 1.0 mm, using a caliper. Sex of individuals was recorded when possible, depending on the stage of gamete development.

RNA/DNA ratios were determined on animals collected every second week during the first month, and once per month thereafter.

RNA/DNA ratios were analyzed using enhanced ethidium bromide fluorescence. The protocol of Kenchington (1994) was used with only slight modification for tissue type. For adductor tissue, centrifugation was performed at room temperature to alleviate jelling of the homogenate which occurred with refrigeration. A Model 112 Turner fluorometer at a setting of 3, excitation of 365 nm, and emission of >570 nm was used for fluorometric readings. Stan-

dard curves were calibrated using standard preparations of calf thymus DNA and yeast RNA. For each adductor sampled, the gonad of the corresponding animal was analyzed. Gonad tissue was also centrifuged at room temperature but RPM's were increased to 3,000 to increase separation of the phases.

All data were ln transformed to correct for skewness and the normality of the data was assessed using the Kolmogorov-Smirnov Goodness of Fit Test (K-S test). Homogeneity of variances was tested using Bartlett-Box or Cochran's tests depending upon the number of observations per cell (sex being the factor for which single observations were possible). Simple correlations were made between selected sets of variables. RNA/DNA and wet weights, for gonad and adductor tissue, were analyzed separately by analyses of variance (ANOVA). Due to a delay in procurement of the last FRDL sample, RNA/DNA ratios were sampled on March 1 from DFO, and March 7 from FRDL scallops. As both of these samples were post-spawn, the data were combined (as day 111) to avoid empty cells in the ANOVA. The ANOVA design tested for the effects of 3 factors (sex, location, and sampling date), and their interactions. Non-significant factors were removed from the designs, and the analyses repeated. The power of the tests was assessed at the 0.05 level.

### RESULTS

The wet weight of the gonad decreased during the second week of conditioning when residual gametes were released or re-absorbed. From this point, at each location, gonad wet weights increased for approximately 95 days (Fig. 1, Table 1). Traces of gametes were observed in the DFO conditioning tank in late January. Broodstock spawned naturally, at least on days 92 and 118 at the government lab and the commercial hatchery, respectively. D-stage larvae were observed 3 days after spawning indicating the production of viable gametes. Analysis of variance showed a significant ( $P = 0.000$ , power 1.00) effect of time on gonad wet weight. Sex and conditioning location were non-significant factors. The average shell height of the broodstock was  $92 \pm 5$  mm, and there was no significant difference in shell height between conditioning locations.

Muscle wet weights differed between conditioning locations ( $P = 0.038$ , power 0.55), with significantly higher meat weights at the commercial hatchery (Table 1). This difference did not de-

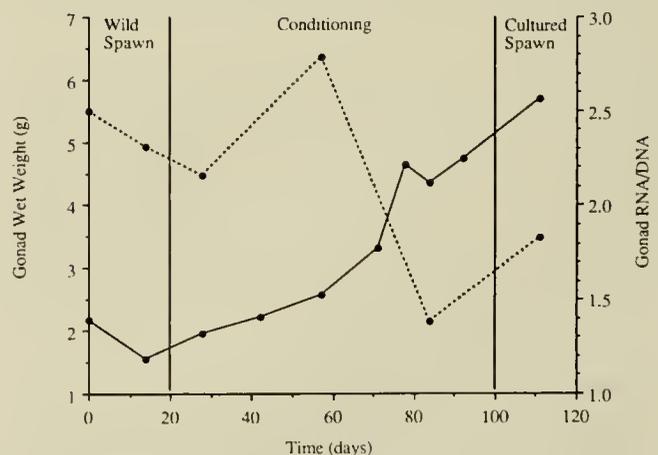


Figure 1. Mean gonad wet weight (solid line) and RNA/DNA ratio (dashed line) of scallops sampled during artificial conditioning. Data from both conditioning locations were combined.

TABLE 1.

Mean values of scallop variables found to vary significantly ( $P = 0.05$ ) in ANOVA of data collected during conditioning.

Variable	Factor	Factor Level	Mean Value	Standard Deviation
Muscle RNA/DNA	Location	Commercial	0.940	0.169
		Hatchery		
		Government		
	Time	Laboratory	0.719	0.202
		14 days	0.700	0.179
		28 days	0.819	0.207
		57 days	0.683	0.104
Gonad RNA/DNA	Sex	84 days	0.941	0.203
		111 days	0.844	0.287
	Time	Male	1.279	0.601
		Female	2.622	0.800
		14 days	2.306	0.641
Muscle Wet Weight (g) (Combined Data)	Location	28 days	2.158	0.575
		57 days	2.782	0.932
		84 days	1.383	0.846
		111 days	1.826	0.726
Muscle Wet Weight (g) (Commercial Hatchery)	Time	Commercial	13.50	2.65
		Hatchery	15.23	4.01
		Government	13.50	2.65
		Laboratory	13.50	2.65
		14 days	11.48	2.03
Gonad Wet Weight (g)	Time	28 days	12.83	2.14
		57 days	15.13	4.99
		84 days	18.12	2.78
		111 days	18.58	2.49
		14 days	1.54	0.53
Gonad Wet Weight (g)	Time	28 days	1.95	0.65
		42 days	2.22	0.57
		57 days	2.57	1.00
		71 days	3.31	2.72
		78 days	4.63	2.19
		84 days	4.34	2.09
		92 days	4.72	1.51
		111 days	5.69	2.17

velop until after approximately 50 days of conditioning (Fig. 2). There was also a significant interaction between location and time ( $P = 0.018$ , power 0.88), however, there was no significant overall effect of sex or time. In order to elucidate the interaction effect, separate analyses of the effect of time on muscle wet weight by location were performed. There was a significant ( $P = 0.004$ , power 0.93) effect of time on meat weight among the hatchery conditioned animals. This effect was not seen in the data from the government lab experiment, hence, the interaction effect observed above. During conditioning, muscle wet weights increased in the hatchery stock (Table 1).

Mean RNA/DNA ratio of the adductor muscle was  $0.780 \pm 0.217$ . Analysis of the cell means showed a significant effect of location ( $P = 0.000$ , power 1.00), time ( $P = 0.001$ , power 0.97), and location-time interaction ( $P = 0.004$ , power 0.90). Scallops at the FRDL hatchery had a higher mean RNA/DNA ratio than those at the government laboratory (Table 1). The peak in the muscle ratio was observed at day 84, just prior to spawning (Fig. 3). There was no significant effect of sex on muscle RNA/DNA ratios. The mean RNA/DNA ratios at the two locations followed the same trend over time with the exception of the post-spawn

sample. The ratio decreased in the final sample from the government lab, while it increased in the same sample from the commercial hatchery. This divergence appears to be the cause of the location-time interaction.

Mean RNA/DNA ratio of the gonad was significantly ( $P = 0.000$ , power 1.00) lower in males than in females (Table 1). There was also a significant effect of time ( $P = 0.000$ , power 1.00) on the ratio, as well as a significant interaction between sampling date and sex ( $P = 0.014$ , power 0.80). Only the female shows a significant difference over time, however, both sexes follow the same trend. The gonad RNA/DNA ratio peaked in the day 57 sample and declined sharply in the subsequent sample at day 84 (Fig. 3). There was a strong ( $r^2 = 0.91$ ) negative correlation between the RNA/DNA ratio of the gonad and the muscle ( $y = 5.86 - 4.72x$ , Fig. 3). There was no significant effect of conditioning location on gonad RNA/DNA ratios.

## DISCUSSION

Wild populations of *P. magellanicus* may take from 4 to 9 months to complete a reproductive cycle, from the initiation of gamete development to spawning. The longer time periods are attributed to early-winter initiation of gonad growth followed by quiescence during the winter months when environmental food supplies are low (Robinson et al. 1981, Gould et al. 1988). By contrast, in some sea scallop populations, the initiation of gonad growth occurs only in the spring, and depends upon the restoration of somatic tissues by energy derived from ingested food, which is more readily available during the spring phytoplankton bloom (Thompson 1977). In a 2-year study of Bay of Fundy scallops, Kenchington et al. (1994) found seasonal variations in gonad and adductor muscle weight. Somatic weight variation indicates either accumulation or utilization (degrowth) of storage substrates (Barber and Blake 1991). Gametogenesis was observed to begin in January with spawning taking place in late August or early September. The cycle of wet-weight changes of the muscle and of the gonad were modeled by sine curves, and the curves for the two tissues were almost exactly out of phase with one another; that is,

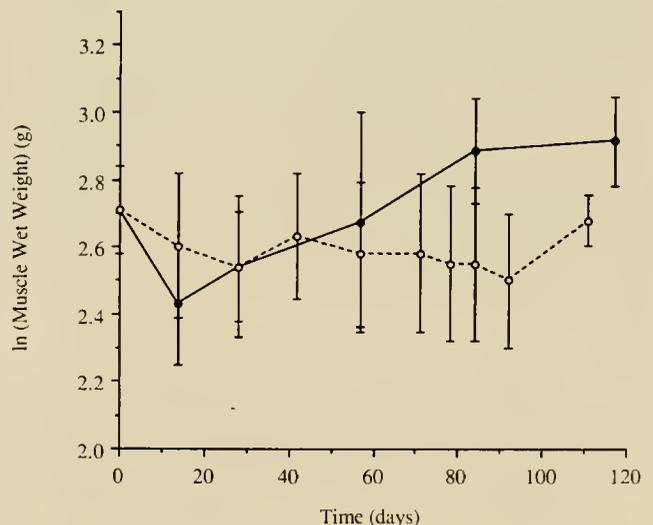
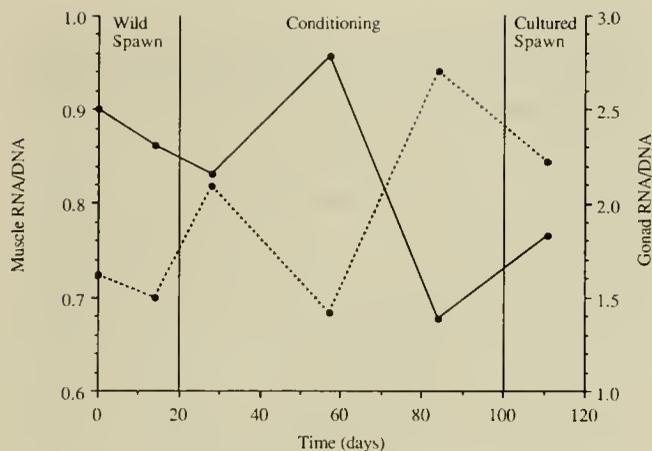


Figure 2. Ln-transformed mean muscle wet weight of scallops sampled during artificial conditioning at a commercial hatchery (solid line) and at a government laboratory (dashed line). The standard deviations are indicated by error bars.



**Figure 3.** Mean RNA/DNA ratios of scallop gonad (solid line) and muscle (dashed line) tissues sampled during artificial conditioning. Data from both conditioning locations were combined.

the wet weight of the adductor muscle declined as the wet weight of the gonad increased.

Like many other bivalves, scallops have seasonal cycles of energy utilization which are associated with the reproductive cycle (Ansell 1974, Robinson et al. 1981, Besnard 1991). During gametogenesis, energy requirements are fulfilled by available food, in conjunction with lipid, carbohydrate, and protein substrates supplied to the gonad (Barber and Blake 1991). The degree to which particular substrates from a specific storage site are used depends on the species and the environmental conditions. In *P. magellanicus*, the adductor is believed to supply the gonad with carbohydrate in the form of glycogen (Gould et al. 1988, Robinson et al. 1981). However, Couturier and Newkirk (1991) also observed the mobilization of protein, and Lubet et al. (1991) have reported the translocation of lipids to the gonad during gametogenesis in the scallop *P. maximus*.

The study of energy utilization during the artificial conditioning of broodstock is an aspect of scallop biology which has received limited attention. Barber and Blake (1991), suggest that somatic weight decline indicates utilization of reserves. However, lack of decline does not necessarily imply the lack of nutrient mobilization from a particular storage site. Reproductive energy metabolism consists of continual energetic exchanges between somatic and reproductive tissues (Faveris and Lubet 1991). In a study of the reproduction of *Placopecten* in suspended culture, Couturier and Newkirk (1991) observed little change in adductor muscle weight during gonad growth. However, during this same period, the chemical composition of the adductor changed considerably. In particular, adductor protein content (as a percent of dry weight) declined sharply from June to August, just prior to spawning.

In the present study, the 3.5-month period observed between the start of laboratory conditioning and the spawning event, represents a contraction of the gametogenic cycle observed in nature in two respects: decreased time span between autumnal spawning and the initiation of the subsequent gametogenic cycle, and decrease in the gametogenic period.

Gonad weights observed in December and March in conditioned animals were comparable to those of scallops of the same average height in a wild Digby, Bay of Fundy, scallop stock (Kenchington, unpublished) in March and August, respectively (comparable developmental phases). Thus there was no detectable

difference in fecundity, as indicated by size-standardized gonad weight, between conditioned- and naturally-developed animals. Gonad development appeared to occur in a two-stage process consisting of an initial period of low variance among individuals, followed by an increase in variance in samples beyond day 42 (Table 1), indicative of individual differences in the rate of gamete development.

Despite the contraction of the gonad development period, a relative constancy of adductor weight was observed. The location of conditioning was a significant factor in the analyses. The broodstock at the commercial hatchery had significantly higher muscle weights than those conditioned in the government lab. However, the mean adductor weight during conditioning at either location was approximately twice as high as those reported for the same sized scallops from the Digby wild stock during the same stage of gonad development (Kenchington et al. 1994). Thus, despite the possible translocation of metabolites from the adductor muscle to the gonad, the consistent supply of a high-quality phytoplankton diet during conditioning may have enabled the adductor muscle to maintain a high average weight through gametogenesis.

RNA is an obligate precursor in protein biosynthesis, and ribosomal RNA (rRNA) normally constitutes > 90% of cellular RNA. The RNA/DNA ratio is a self-calibrating index of protein synthesis, and in mammalian experiments is seen to increase prior to protein synthesis (Reeds 1987). Due to the role protein plays in the synthesis of other materials, variation of RNA/DNA is considered reflective of cell growth (Brachet 1960, Pease 1976). By recording levels of RNA/DNA in tissues sampled during conditioning, it is possible to infer changes in metabolic activities, particularly protein synthesis, associated with gametogenesis.

RNA/DNA ratios fell within the range of values recorded in the literature for bivalve adductor muscle (e.g., Pease 1976, Kenchington 1994) and gonad (Robbins et al. 1990, Martinez et al. 1992). One of the most interesting observations of this study was the strong negative correlation between the gonad and adductor muscle RNA/DNA ratios. While this data is only correlative, it implies that protein synthesis in the muscle is related to protein synthesis in the gonad, and that this relationship is "on-off" in nature (Barber and Blake 1991), suggestive of neurosecretory involvement. One explanation of this observation is a hormonal suppression of protein synthesis in the muscle during gonad protein synthesis. If such a mechanism occurs, it would ensure that when protein synthesis is required for gametogenesis or gonad maintenance metabolism, digestion products are preferentially used by the gonad.

Significant effects of time and location were seen in the ANOVA analyses of the RNA/DNA ratios of the adductor muscle. The mean values observed at each sampling location showed a peak in the ratio prior to spawning. The ratios were also higher in animals from the commercial hatchery, reflecting the significantly higher mean wet weight observed at that location. In the absence of information on the conditioning protocol used at the commercial hatchery, the causes of location-specific differences can only be speculative, but are most likely related to food quality or quantity.

In contrast, the gonad RNA/DNA ratios showed significant differences between sex and time, but not between locations. As in the muscle RNA/DNA, the gonad ratio changed little during the first 8 weeks of conditioning. When conditioning the Chilean scallop, *A. purpuratus* in lab and suspended culture, Martinez et al. (1992) noted an early DNA increase in the gonad, apparently due to proliferation of oogonia. At this time, the RNA/DNA ratio changed little, as the RNA content also increased, thus maintain-

ing the proportion. A similar process may have occurred in *P. magellanicus*, as gonad weight increased despite the constancy of the ratio during the first 8 weeks. However, in the day 84 sample (between weeks 8 and 12 of gametogenesis) a significant decline in gonad RNA/DNA occurred (for both sexes).

This decline in gonad ratio was concurrent with both an increase in muscle RNA/DNA (Fig. 3) and a significant gonad weight increase (Fig. 1). The nature of the gonad RNA/DNA decline (gradual or abrupt) over the 1-month interval is not known. A decline in RNA/DNA ratio in the gonad tissue of hatchery-held Chilean scallops, was also observed, however, this was attributed to insufficient food availability (Martinez et al. 1992). During the conditioning of *P. magellanicus*, food was not a limiting factor. Pease (1976) also noted a seasonal autumnal decrease in the RNA/DNA ratio of soft body tissue of the oyster *C. virginica*, which he attributed to variation in the presence of gonad tissue in the mantle. Given the corresponding increase in muscle RNA/DNA, this phase of development may represent a significant shift in the internal mobilization of energy. Robbins et al. (1990) observed a decline in gonad ratio (for both sexes) as gonad maturity proceeded. They identified a relationship between the level of aspartate transcarbamylase (ATCase, E.C. 2.1.3.2) activity and RNA/DNA variation, both of which were minimal prior to spawning. The ATCase enzyme is involved in "de novo" biosynthesis of pyrimidine bases, and has been used as a gametogenic indicator in bivalves (Bergeron and Buestel 1978).

Sexual differences were identified in the mean gonad RNA/DNA ratio, with the ratio being higher in female scallops. In a seasonal study of the biochemical variation in a wild population of the hermaphrodite scallop *P. maximus* (Robbins et al. 1990), female ratios were consistently ten times greater than male ratios. The elevated ratios reflected the production, by males, of a large

number of small gametes, as compared to the female production of fewer, larger ova. Therefore, males contain a greater number of nuclei, and ultimately, a higher DNA content.

According to Naidu (1970), spermatogenesis occurs more rapidly than oogenesis in Newfoundland scallops. However, in the present study with Nova Scotian stock, we observed DNA values to be higher in males beginning on January 5. Earlier samples appeared to have smaller values of DNA and RNA in males, as compared to females, reflecting perhaps a slower development in males. This corresponded to macroscopic observations which permitted female identification before males.

In conclusion, it would appear that practices currently used to condition sea scallops provide more exogenous food than is required by the animal. The scallops in both hatcheries had sufficient food, at the selected temperature, to undergo gametogenesis, and to maintain a condition of the muscle beyond that which occurs in the wild. As food production is a costly and often limiting aspect of hatchery operations, it may prove beneficial to manipulate the use of endogenous reserves to produce conditioned broodstock with less food. Current concepts of algal requirements may also warrant revision.

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## REPRODUCTIVE CYCLE OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791), ON NORTHEASTERN GEORGES BANK

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**ABSTRACT** Autumn (September to October) has typically been assumed to be the sole spawning period for the sea scallop (*Placopecten magellanicus*) population on Georges Bank. The present study establishes the occurrence of a semiannual reproductive cycle, with spawning in the spring and autumn. Scallops collected over a seven-year period from research and commercial cruises were examined histologically to determine mean oocyte diameter and gamete volume fraction (GVF) and analyzed for gonosomatic index (GSI). Sampling for spring-spawned sea scallop larvae was carried out in May 1991. GSI data revealed a bimodal pattern in gonadal development characteristic of a semiannual spawning cycle. Data suggest that the largest and most synchronized spawn occurred in the late-summer, early-autumn (September–October) after which scallops were reproductively spent. The spring-summer (May–June) spawn, dependent on gametogenesis initiated in autumn, was temporally erratic, not occurring at all in 1989 or 1992, and characterized by a more protracted spawning period than the autumn. The onset, duration and periodicity of spawning seemed more consistent interannually in autumn than in spring spawns. Although the GSI gave a rapid and accurate assessment of reproductive activity, histological techniques allowed for direct cytological observations of gametes and thus provided more detailed information. Qualitative and quantitative histological observations confirmed the presence of mature, potentially viable oocytes by early spring (February–March 1991). The presence of planktonic larval stages by May 1991 is evidence that these oocytes were released. GVF data also indicated the presence of resorbing oocytes throughout the majority of the gametogenic cycle. This suggests a mechanism by which sea scallops may recover some proportion of energy allocated to the production of oocytes which will not be spawned. Finally, implications a semiannual reproductive strategy could have on the Georges Bank commercial scallop fishery are considered.

**KEY WORDS:** Semiannual reproduction, gametogenic cycle, gonosomatic index, scallop

### INTRODUCTION

The sea scallop, *Placopecten magellanicus* (Gmelin 1791), is a commercially important bivalve mollusc found exclusively in the western Atlantic Ocean along the coast of Canada and the United States. Its commercial distribution is defined as the Strait of Belle Isle, Newfoundland to Cape Hatteras, NC (Posgay 1957, Porter 1974). In northern regions sea scallops are found in water as shallow as a few meters, while in southern locations they are confined to waters deeper than 50 m. *P. magellanicus* often occur in localized, dense aggregations referred to as beds. The largest of these beds support commercial fisheries off the coast of Newfoundland, in the Bay of Fundy, the Gulf of Maine, along the mid-Atlantic Shelf, and especially on Georges Bank, the largest natural scallop stock and fishery in the world (Caddy 1989). While inshore scallop populations have been well-studied (e.g., Beninger 1987, Giguère et al. 1994, MacDonald and Thompson 1985a,b, 1986, 1988, Naidu 1970, Parsons et al. 1992, Thompson 1977, Thompson and MacDonald 1990 and references therein), recently only limited scientific consideration has been given to the Georges Bank population (Thouzeau et al. 1991, Tremblay and Sinclair 1990a,b, Tremblay and Sinclair 1992, Tremblay et al. 1994) and other offshore scallop beds. Results of studies on inshore populations may not be relevant for Georges Bank because both growth and reproductive cycles have been shown to differ between inshore

and offshore populations (Barber et al. 1988) as well as among latitudinally separated populations (MacDonald and Thompson 1988).

Until recently, scientific literature suggested that *P. magellanicus* had an annual reproductive cycle with a single spawning period during the late summer or early autumn. Posgay and Norman (1958) reported a short, synchronized autumn spawn of scallops at one site on Georges Bank where they observed that >92% of all males and females examined had progressed from a ripe to a spawning or spent stage of development in the course of just 5 days (September 21 to 25, 1956). Subsequently, observations have generally reported more protracted spawning events characterized by spatial and interannual variability. MacKenzie et al. (1978) used the gonadal condition of Georges Bank scallops to suggest that they were in the progress of spawning in late September through to early October. Langton et al. (1987) suggested that *P. magellanicus* in the Gulf of Maine may ‘switch’ between synchronous or protracted spawnings between years, depending upon specific environmental conditions such as food availability and temperature. Most recently, however, several studies have shown that *P. magellanicus* may have an additional spawning period during the spring, preceding the reported autumn spawn. Naidu (1970) and Barber et al. (1988) have each reported a potential minor spring spawn for scallop populations off the southwest coast of Newfoundland and the Gulf of Maine, respectively. Squires (1958) reported that scallops off Newfoundland were well-advanced towards maturity by mid-June, a fact Naidu (1970) confirmed through histological examinations. DuPaul et al. (1989),

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Schmitzer et al. (1991), and Kirkley and DuPaul (1991) reported a semiannual gametogenic cycle characterizing sea scallops from the mid-Atlantic Shelf, with gonadal development in the spring comprising a longer period of time and resulting in greater fecundity than during the autumn period. Much less is known of reproduction in the Georges Bank population despite their commercial significance.

A semiannual spawning cycle may have important implications for presently employed management strategies which assume an autumn spawn only. An improved understanding of the sea scallop's reproductive cycle and population dynamics is critical for optimizing exploitation from this resource. The primary objective of this study was to describe the gametogenic cycle of the sea scallop population on the northeastern portion of Georges Bank, and to assess the likelihood of a semiannual gametogenic cycle. Histological analyses of gonads, calculation of gamete volume fractions, and measurement of oocyte diameters on scallops collected monthly from May 1990 to May 1991 were performed to assess the stages and potential viability of female gametes. In addition, calculation of temporal changes in gonadal weight relative to body size (gravimetric gonadal indices), on scallops collected between 1984 and 1990, permitted assessment of intra- and inter-annual variability in reproductive cycles. Finally, a limited number of plankton tows were conducted in the spring of 1991 in an attempt to sample sea scallop larvae.

## MATERIALS AND METHODS

### Sampling

Sea scallops were opportunistically sampled from the Canadian side of Georges Bank during the period of May 1990 to May 1991. Two sets of samples were collected aboard the FRV EE Prince (Bedford Institute of Oceanography) during the months of May and August, 1990 (hereafter referred to as research cruise samples). Because vessel time was limited, samples for the remainder of the year were obtained from several commercial scallop draggers (hereafter referred to as commercial samples). Research cruise data represent intensive sampling of a 12-station grid distributed over the northeastern portion of Georges Bank (Fig. 1). These data were analyzed to delineate spatial variation in reproductive characteristics. Each commercial sample represents a single station sampled per month at different locations, but over the northeastern portion of the Bank (Fig. 1). Commercial samples collected monthly between May 1990 and May 1991 were used to consider temporal differences in gametogenesis of sea scallops. All samples were collected from depths between 66 and 102 m. A rigid spatial-temporal sampling strategy was not possible due to variation in commercial fishing activity.

### Assessment of the Gametogenic Cycle

#### Gonosomatic Index (GSI)

Reproductive development was initially quantified by a gonosomatic index (GSI), here defined as the ratio of fresh gonad weight to fresh total soft body weight \* 100 (Giese and Pearse 1974, deVlaming et al. 1982). Thirty scallops ( $\geq 85$  mm shell height, the distance between the dorsal and ventral margins) were randomly selected from each commercial and research cruise station sample. For each individual, the soft body components were divided into somatic and gonadal tissues. The crystalline style was removed from the gonad and included with somatic tissues. All tissues were drained, blotted dry, and weighed to the nearest 0.01

g. Data for males and females were combined to calculate mean monthly GSI values because no significant differences in standardized gonadal tissue weight (GSIs) could be detected in any of three months (i.e., April  $F_{1,58} = 1.736$ ,  $p = 0.193$ ; June  $F_{1,88} = 2.952$ ,  $p = 0.089$ ; September  $F_{1,88} = 0.119$ ,  $p = 0.905$ ) randomly selected from 1984 of the 1984–1992 GSI data set.

To analyze multi-year trends in reproduction, the data collected in 1990 and 1991 were coupled to a GSI data set already established for Georges Bank covering the period 1984 to 1992. The additional GSI data was collected using the same sampling protocol employed for 1990 and 1991 data (e.g., 1:1 female ratio where possible,  $\geq 85$  mm shell height, etc.). All samples were collected from the northeastern portion of Georges Bank at depths ranging between 60 to 120 m. May and August samples were collected on research cruises (i.e., stock assessment cruises) by G.R. while the remaining months were sampled by the commercial fleet. Note in Figure 3 that eight out of the 108 month time series (i.e., March 1984, December 1984 and 1985, January 1985 and 1986, February 1985 and September 1988 and 1989) were not sampled by the commercial fleet. Examination of multi-year baseline data was critical for accurate assessment of the interannual variability in spawning and reproductive development.

### Histological Analyses

Thirty live, mature scallops (15 males and 15 females) were randomly selected from each station of commercial and research cruise samples for histological studies. Shell height of each individual was measured to the nearest mm using vernier calipers. Gonads were resected from each animal and sex was recorded. The presence of gametes within the gonad were obvious as oocytes were red while sperm were beige. A proximal and distal portion of each gonad was sampled and fixed in Bouin's solution (prepared according to Humason 1979). Following a minimum of 72 hr in fixative, all tissues were preserved in 70% ethanol until further processing. Subsequently, tissues were dehydrated through an ascending alcohol series, cleared in xylene and embedded in paraffin wax. Tissues were sectioned (6 to 8  $\mu$ m) on a rotary microtome, mounted on slides and counter stained with Harris' hematoxylin and eosin-yellow.

*Oocyte Diameter.* Mean oocyte diameters, mature and developing eggs, of 15 females ( $n = 30$  oocytes per individual) per station sampled (research and commercial cruise samples) were determined from histological sections using a compound microscope fitted with an ocular micrometer (200 $\times$ ). Only oocytes sectioned through the nucleus (i.e., near the egg's center = maximum diameter) were measured.

*Gamete Volume Fraction (GVF).* Gamete volume fractions were derived from point count estimates made with a Weibel test grid applied to randomly selected fields of view of thin tissue sections according to MacDonald and Thompson (1986). Point count estimates were converted to proportions of the gonad occupied by developing gametes (DGVFs), mature gametes (MGVFs), resorbing or atretic gametes (RGVFs) and total gametes (TGVFs = DGVFs + MGVFs + RGVFs). Other cytological features observed included inter- and intrafollicular space, connective tissue and a miscellaneous group of unidentified tissues.

Histological observations revealed that males possessed considerable numbers of spermatozoa within follicles and evacuating ducts several weeks before females were observed to have oocytes within evacuating ducts. Males appeared to have the ability to spawn over a longer period of time relative to females. If so, then

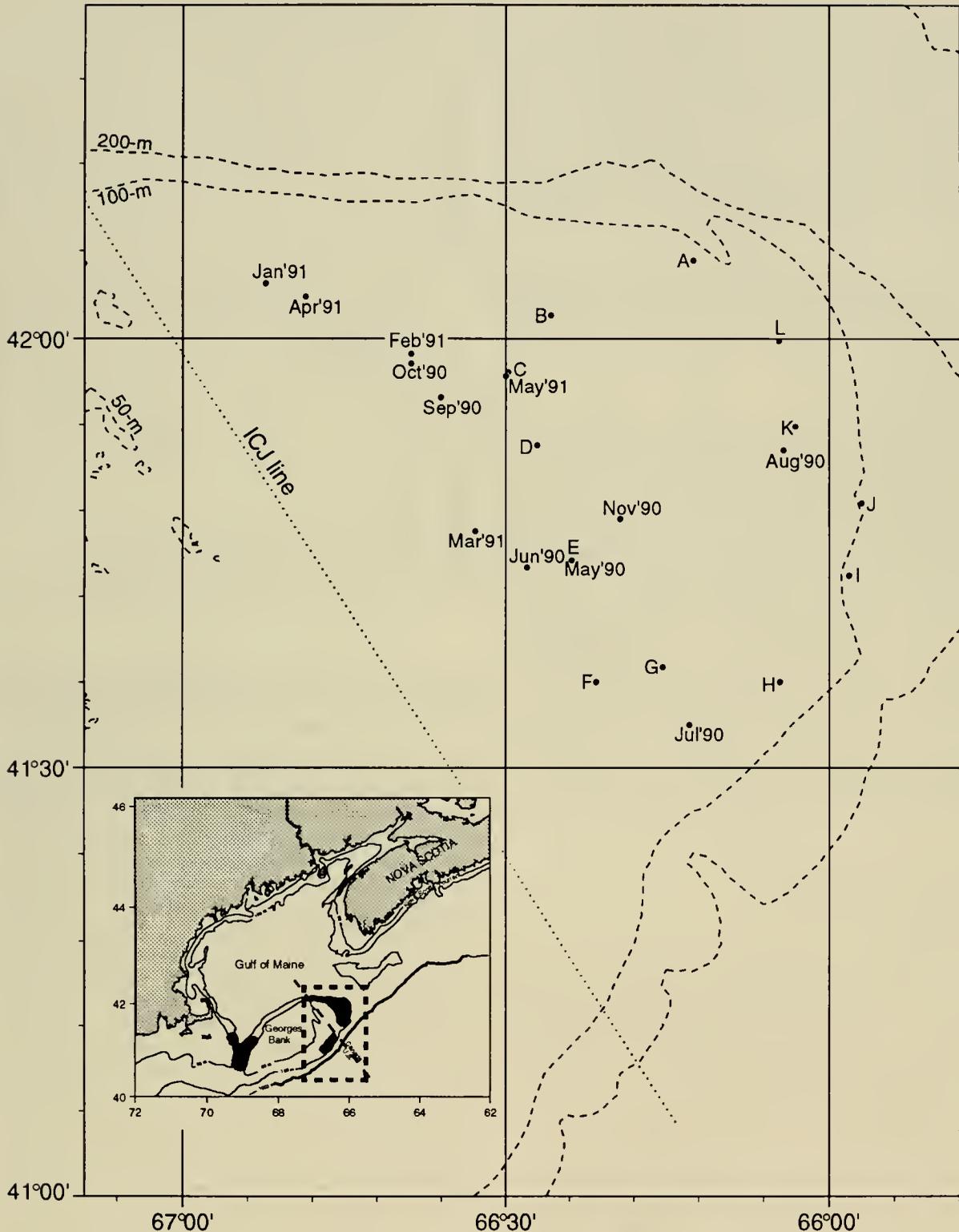


Figure 1. Location of the 12 station grid (see stations A to L) on the northeastern section of Georges Bank sampled during research cruises conducted in May and August, 1990. Also, the location of monthly commercial cruise samples collected over northeastern Georges Bank between May 1990 and May 1991 (see stations indicated by month). The 'International Court of Justice' (ICJ) line defines the boundary between Canadian and US waters. Inset: Georges Bank in relation to the east coast of Canada and the US.

female development would determine when a viable spawn may occur by releasing mature oocytes. Thus, only females were examined to delineate semiannual spawning cycles.

A set of descriptive criteria, in accordance with the observa-

tions of Naidu (1970) and Davidson and Worms (1989), were used throughout the study to distinguish between developing and mature oocytes. Preliminary analyses revealed mature oocytes to be approximately 55 to 60  $\mu\text{m}$  in diameter or larger with considerable

vitelline surrounding a distinct nucleus. Lack of vitelline was typical of developing oocytes, even though a well-defined nucleus may be present. Resorbing oocytes were easily detected by the loss of the rounded or polygonal shape characteristic of mature oocytes. Oocytes of seven individuals were examined per station with five randomly selected fields of view per individual being quantified. Each field ( $\sim 0.32 \text{ mm}^2$ ) contained 42 points. Statistical analyses were carried out on tallied point counts of gamete volume fractions estimated per individual, while corresponding percentage data were presented in figures for clarity of comparison.

Statistical analyses were performed on oocyte diameter and GVF data using general linear models of analysis of variance. Data were checked for normality and homogeneity of variance, no transformations were required as the data did not violate either of these assumptions.

#### Zooplankton Sampling

Four stations (i.e., four tows) located on the northeastern section of Georges Bank were sampled to test for the presence of planktonic scallop larvae, from the suggested spring spawning period, on 23 May 1991 (Fig. 1). Limited funding and shiptime precluded more extensive sampling of the zooplankton. Larvae were sampled with twin, 40-cm diameter, vertical bongo nets fitted with 58- $\mu\text{m}$  and 85- $\mu\text{m}$  nitex plankton nets. Plankton nets were lowered to within 5 to 10 m of the bottom and towed to the surface at a speed of approximately  $1 \text{ m} \cdot \text{sec}^{-1}$ . Samples were preserved in 4% formalin buffered with sodium borate. The physical separation of scallop larvae and other heavier components of the plankton samples was conducted in the laboratory using a colloidal silica (Tremblay et al. 1987). Sea scallop larvae were then identified, counted and measured under a dissecting microscope. All length measurements (along the longest axis parallel to the hinge) were made to the nearest 5.8  $\mu\text{m}$  with the aid of an ocular micrometer fitted to an inverted microscope.

## RESULTS

#### Assessment of the Gametogenic Cycle

##### Gonosomatic Indices

Gonosomatic indices estimated from research cruise stations (A–L) revealed significant differences among the sampled locations in May (Fig. 2A;  $F_{11,348} = 3.090$ ,  $p < 0.001$ ) and August (Fig. 2B;  $F_{11,348} = 21.341$ ,  $p < 0.001$ ) 1990. This suggests that there are spatial differences in GSI data over the sampled region of Georges Bank. The spatial variability appears to be larger in August (Fig. 2B) than May (Fig. 2A) and it also appears to be as large as the temporal variability observed in both these times of year. For example, the range of GSI values estimated for stations sampled in May and August 1990 (approximately 4% and 10%, respectively) are comparable to the temporal ranges observed from April to May (i.e., minor spring spawn) and September to October (i.e., major autumn spawn) (Fig. 3). Although this precludes definitive statements being made from temporal data presented in Figure 3, this data can be used to highlight potential spawning periods to be examined with more rigorous techniques (i.e., see histological data below).

Typically, the GSI was observed to increase between December and February, indicative of early stages of gametogenesis (when gametes were differentiating but not yet showing signs of

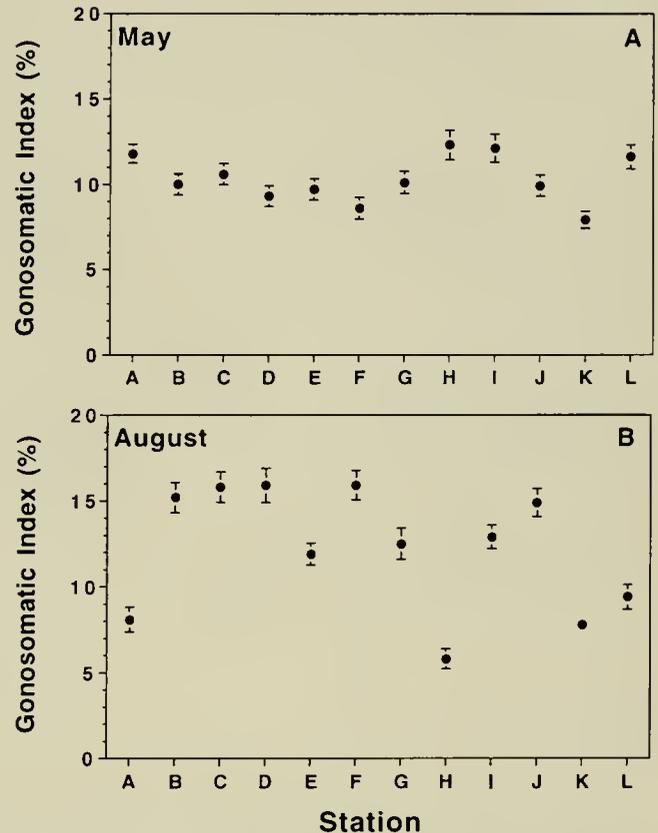


Figure 2. Mean gonosomatic index (GSI) for sea scallops sampled from stations A–L (see Fig. 1) on northeastern Georges Bank in (A) May 1990 and (B) August 1990. Vertical lines represent  $\pm 1$  SE.

growth). A more rapid increase in the GSI was observed in February and peaked in April or May. The index subsequently dropped over a short period of time (usually by June or July) which may have indicated a partial spawn. This was followed by a second increase in the GSI which peaked in August or September. The largest decrease in GSI occurred as a result of the major autumn spawn after which the GSI was annually lowest in October or November (Fig. 3) when gonads were spawned out. A resting period of several months was observed twice following the autumn spawn (see late-autumn, early-winter of 1989–90 and 1990–91).

The magnitude of the spring and autumn spawning periods, estimated by observing the extent of the drop in the GSI between a peak and the subsequent trough, varied interannually, particularly for the spring spawn. An examination of GSI data, spanning 1984 through 1992, most strongly suggests a spring and autumn spawning episode for 1986 and 1991. Although the autumn spawn is apparent in every year, the spring spawn becomes more questionable in 1984, 1985, 1987, 1988 and 1990, while there is no apparent spring spawn in 1989 or 1992 (Fig. 3). The relative difference between the spring and autumn spawns also varied between years. This was apparent from the two distinct spawns characterizing 1986, the minor double peak spring spawn of 1988 observed prior to the autumn spawn, as well as the lack of a spring spawn in 1989. The magnitude of the autumn spawn was consistently larger than the spring spawn (Fig. 3).

#### Histological Analyses

*Oocyte Diameter.* Analyses of variance was used to examine spatial and temporal variation in oocyte diameters from research

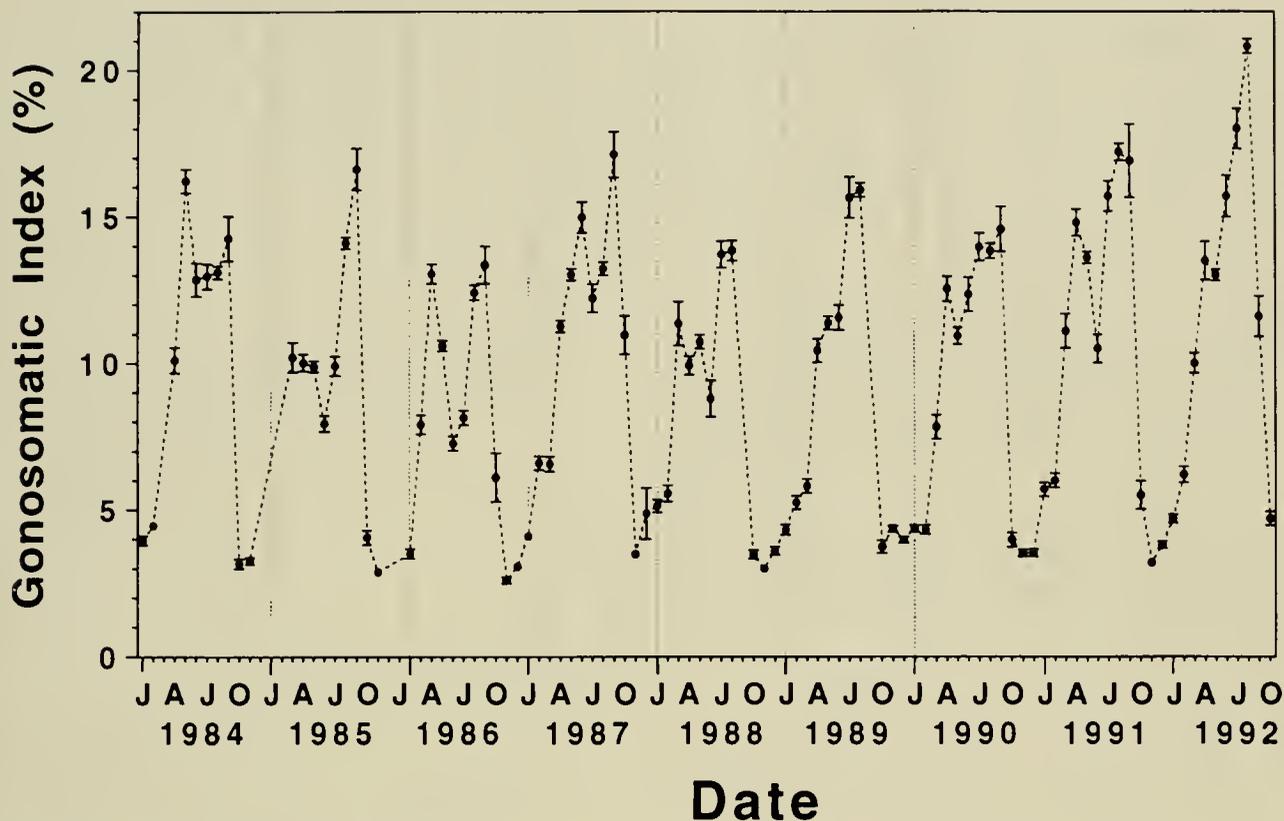


Figure 3. Mean monthly gonosomatic index (GSI) for sea scallops sampled over northeastern Georges Bank between 1984 and 1990. Vertical lines represent  $\pm 1$  SE.

and commercial cruise samples, respectively. There were significant differences in egg diameter among sampled research cruise locations in both May (Fig. 4A;  $F_{11,156} = 12.297$ ,  $p < 0.001$ ) and August (Fig. 4B;  $F_{11,108} = 11.306$ ,  $p < 0.001$ ). Thus, some variation in egg size was attributed to regional differences over the sampled portion of Georges Bank. These differences, however, were mostly small relative to temporal variation observed in oocyte diameter (see below). The mean oocyte diameter at station H in August 1990 (Fig. 4B) was considerably lower than all other stations. Histological observations revealed that spawning had started at this station. The larger, mature oocytes had been released while the smaller, immature oocytes still present accounted for the decrease in mean oocyte diameter.

Oocyte diameter per commercial station varied significantly among months ( $F_{11,156} = 444.167$ ,  $p < 0.001$ ). September 1990 represented the last collection prior to the initiation of the autumn spawn and scallops collected at this time possessed the largest, most mature oocytes. Oocytes sampled in August and October 1990 from developing and partially spent gonads, respectively, were smaller than those observed in September. May and July 1990 samples exhibited the smallest estimates of mean oocyte diameters with the exception of November 1990 and January 1991 when no mature oocytes were observed within the gonads. Mean oocyte diameters sampled in June 1990 were larger than those sampled in both May and July 1990, however, the difference in oocyte diameter was comparable to the range observed in the spatial data (Fig. 4A,B). Patterns observed in these data were consistent with the gonosomatic indices and histological observations which suggest a minor spring spawn, followed by a stage

where more oocytes matured in preparation for an autumn spawn. Spring gametogenesis was typically initiated following the autumn spawn and continued through to June for 1990, while gametogenesis occurred more quickly prior to the autumn spawn.

Mean oocyte diameter estimates were lowest in November 1990 and January 1991 (Fig. 5). Values were considerably larger in February 1991 than in either of these two months, suggesting considerable development early in the year. March and April 1991 values were comparable, but did differ considerably from those sampled in February 1991, again indicating considerable growth of oocytes. Oocyte diameters in May 1991 exhibited an increase from March and April and no apparent difference from the September 1990 peak. This suggests that gonads sampled in the spring of 1991 contained mature oocytes of comparable size and potential viability as gonads sampled during the previous autumn.

*Gamete Volume Fraction (GVF)*. Statistical analyses were presented only for MGVF data as they were considered the best estimate of reproductive potential for sea scallops. Mature gametes were considerably larger in size and more abundant than developing and resorbing oocytes and tend to dominate the limited space available within follicles of the gonad even when present only in moderate concentrations. The abundance of mature gametes, therefore, has a direct effect on stereological estimates made for developing and resorbing oocytes irrespective of their true abundances. However, relevant information remains in these plotted data (see below) particularly when compared with qualitative observations of mature, developing and resorbing oocytes made from histological slides.

Total and mature GVFs estimated from research cruise stations

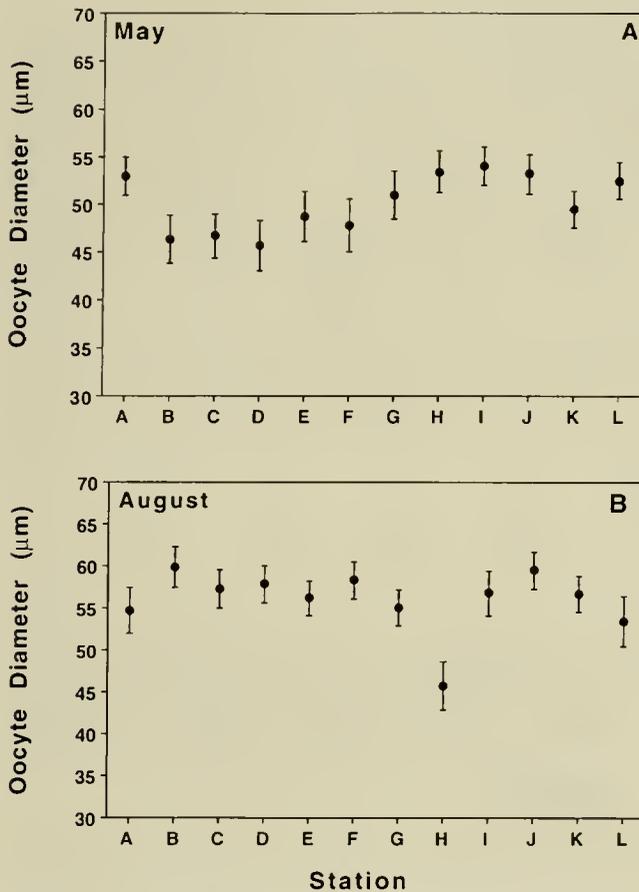


Figure 4. Mean oocyte diameters for sea scallops sampled from station A-L (see Fig. 1) on northeastern Georges Bank in (A) May 1990 and (B) August 1990. Vertical lines represent  $\pm 1$  SE.

(A-L) sampled in May and August 1990 are presented in Figures 6(A,B) and 7(A,B). Analysis of variance revealed significant differences in MGUVs among sample locations both in May ( $F_{11,72} = 30.77$ ,  $p < 0.001$ ) and August ( $F_{11,72} = 41.37$ ,  $p < 0.001$ ) 1990. Similar to the results from the GSI data, the observed spatial variation in MGUVs over the sampled portion of Georges Bank (Fig. 7A,B) was comparable to temporal variability shown in Figure 9. August 1990 MGUV samples showed exceptionally high

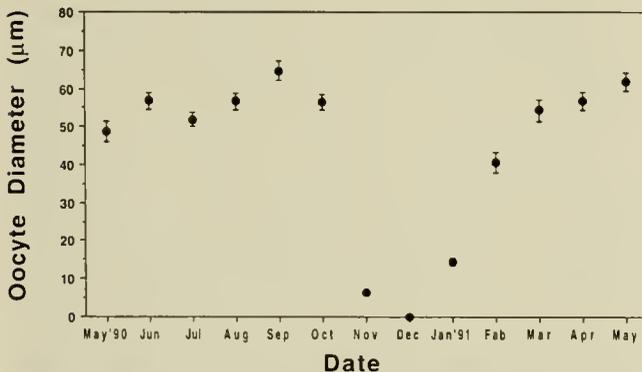


Figure 5. Mean monthly oocyte diameters for sea scallops sampled between May 1990 and May 1991 (see Fig. 1) on northeastern Georges Bank. Vertical lines represent  $\pm 1$  SE.

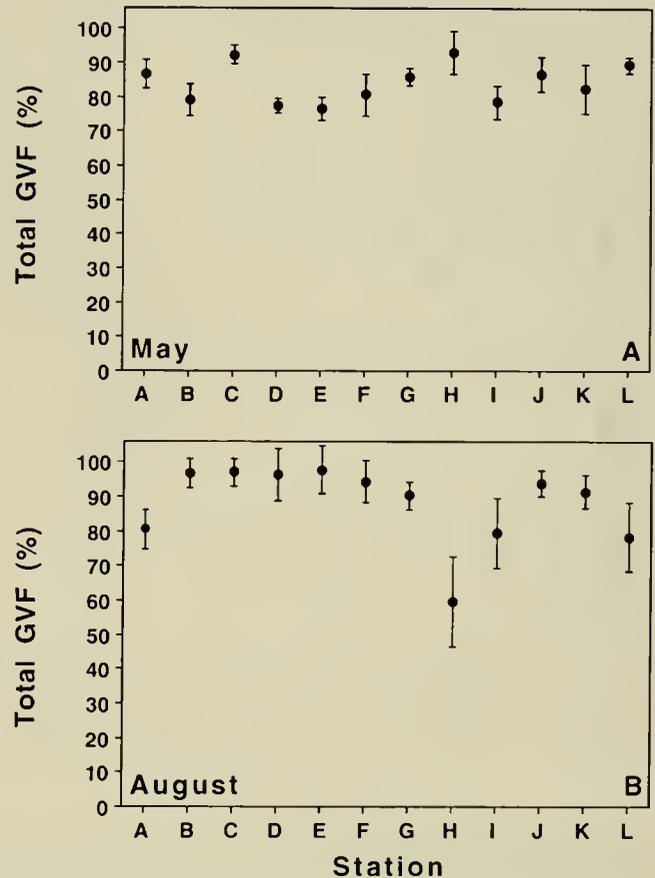


Figure 6. Total gamete volume fractions (TGUVs), expressed as percentage data, for sea scallops sampled from stations A-L (see Fig. 1) on northeastern Georges Bank in (A) May 1990 and (B) August 1990. Vertical lines represent  $\pm 1$  SE.

spatial variability with stations A, G, H, I, and L showing considerably lower values than the remaining stations (Fig. 7B). The low value at station H was again attributable to scallops having initiated spawning prior to collection, as observed above when measuring oocyte diameters. The remaining stations with low MGUVs also showed signs of spawning as well as considerable proportions of follicles filled with resorbing oocytes. In general, scallop gonads sampled in August 1990 typically possessed larger MGUVs than their counterparts sampled in May 1990. Since only mature oocytes were released during a spawn, this supports the observation made earlier from GSI data (Fig. 3) that the autumn spawn was larger than the spring spawn. Nonetheless, MGUVs seen in the May samples were evidence of spawning readiness in spring.

Temporal variability in TGUV (Fig. 8) appears greater than spatial variability (Fig. 6A,B). Maximum values of 80 to 90% for TGUV were observed from June through to September 1990 (Fig. 8). A drop in TGUVs occurred between September and October 1990, corresponding to the autumn spawning period observed from GSI data (Fig. 3). Minimum values of approximately 10 to 20% for TGUVs were observed in October and November 1990 (Fig. 8). The initial increase in TGUV following the autumn spawn occurred immediately between November 1990 and January 1991 (Fig. 8). A rapid increase in TGUV was observed between January and February 1991. A TGUV comparable to values observed in September 1990, just prior to the autumn spawn, was estimated for

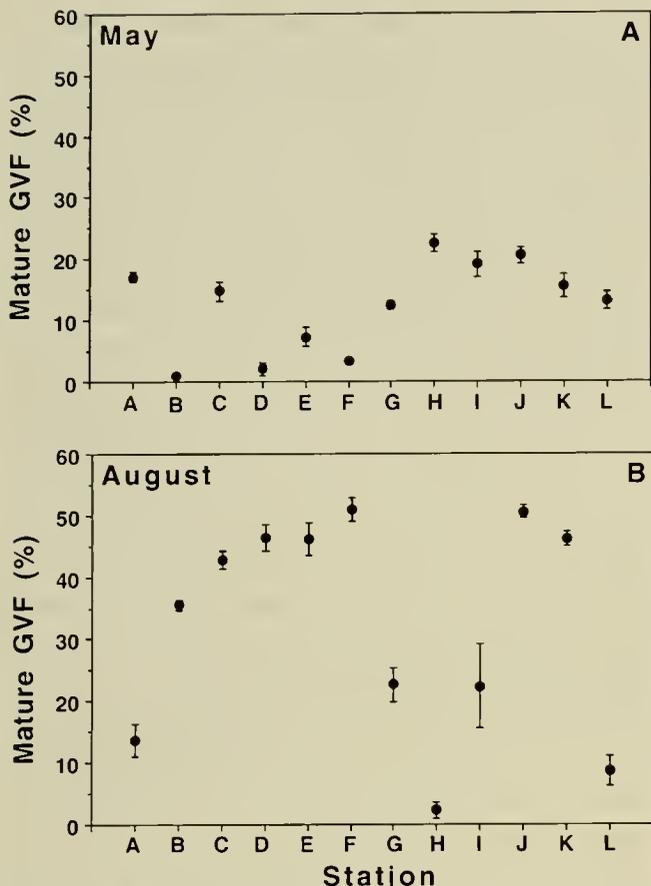


Figure 7. Mature gamete volume fractions (MGVFs), expressed as percentage data, for sea scallops sampled from stations A-L (see Fig. 1) on northeastern Georges Bank in (A) May 1990 and (B) August 1990. Vertical lines represent  $\pm 1$  SE.

March 1991 and remained high until the end of sampling in May 1991.

Mature oocytes were observed throughout the majority of the gametogenic cycle (10 months out of the 13-month sampling period, May 1990–May 1991), but none were observed from November to January (Fig. 9) and a significant difference in MGVF estimates was observed over the sampling period ( $F_{11,72} = 28.80$ ,

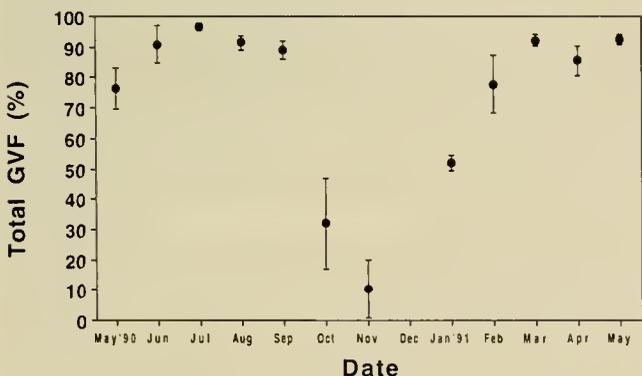


Figure 8. Total gamete volume fractions (TGVFs), expressed as percentage data, for sea scallops sampled between May 1990 and May 1991 (see Fig. 1) on northeastern Georges Bank. Vertical lines represent  $\pm 1$  SE.

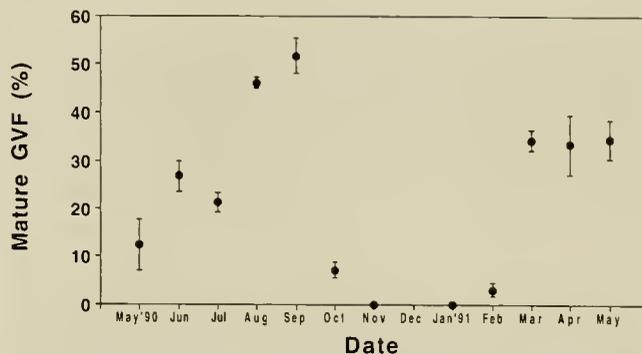


Figure 9. Mature gamete volume fractions (MGVFs), expressed as percentage data, for sea scallops sampled between May 1990 and May 1991 (see Fig. 1) on northeastern Georges Bank. Vertical lines represent  $\pm 1$  SE.

$p < 0.001$ ). Approximately 12% of the gonad consisted of mature oocytes in May 1990 (Fig. 9). MGVFs increased through the summer, peaking in September 1990, just prior to the autumn spawn (Fig. 9). MGVFs were zero in November 1990 and January 1991 (Fig. 9). Mature oocytes were initially observed in February 1991 with considerable proportions of the gonad (approx. 35%) becoming filled by March 1991 (Fig. 9). This continued development and growth of gametes within the gonad corresponded to the increase in TGVFs observed over the same time period (Fig. 8).

Developing gamete volume fractions tended to decrease as the

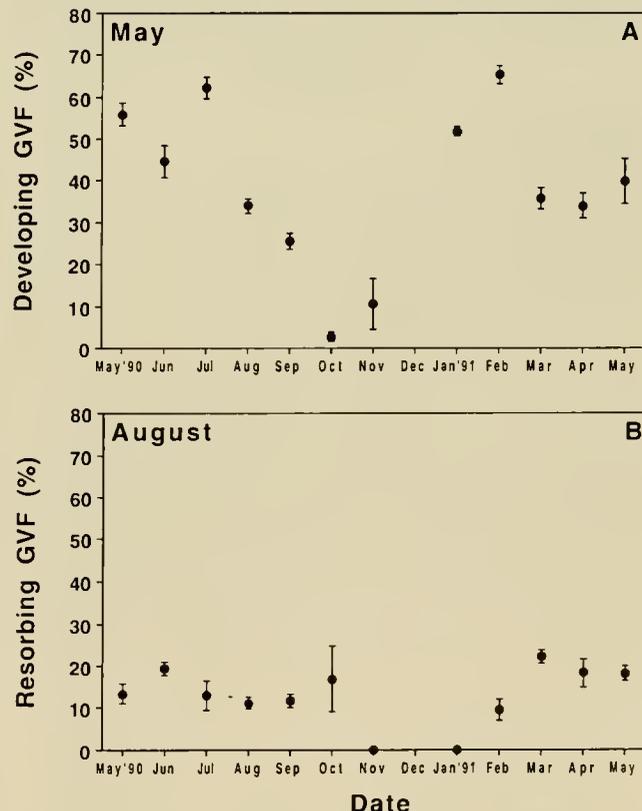


Figure 10. (A) Developing and (B) resorbing gamete volume fractions (DGVF and RGVFs), expressed as percentage data, for sea scallops sampled between May 1990 and May 1991 (see Fig. 1) on northeastern Georges Bank. Vertical lines represent  $\pm 1$  SE.

autumn spawn approached (Fig. 10A). Minimal values were observed following the autumn spawn in October and November 1990, with November showing an apparent increase in DGVs (Fig. 10A). Even though this limited increase could not be attributed to temporal variability alone, qualitative observations revealed the differentiation of oogonia from germ cells. This supports the immediate reinitiation of gametogenesis following the autumn spawn. Developing GVs increased from November through to February 1991 (Fig. 10A) due to the production of more oogonia as well as the development and growth of oogonia and immature oocytes. The large decline between February and March was due to the corresponding increase in MGVs (Fig. 8). The larger mature gametes occupy more space and subsequently cause a decrease in the proportion of developing gametes observed on the stereological test grid. This influence of mature gametes on the relative proportions of developing gametes observed precluded the use of statistics to test temporal variations.

Qualitative observations from histological sections revealed only mature oocytes undergoing resorption. Therefore, aside from those months when mature oocytes were not present (Fig. 9), resorbing oocytes were consistently present (Fig. 10B) with estimated volume fractions varying between approximately 10 to 20%.

#### Zooplankton Sampling

Sea scallop larvae were present, but were not abundant at all 4 sampled stations (stations C–E, Fig. 1). Mean shell lengths ( $\pm$ SD, N) observed for stations C through E were 184  $\mu$ m ( $\pm$ 38.47, 5), 153  $\mu$ m ( $\pm$ 41.63, 3), 155  $\mu$ m ( $\pm$ 30.00, 4), and 140  $\mu$ m ( $\pm$ 20.00, 3), respectively.

### DISCUSSION

#### Reproductive Events

The bimodal distribution of the GSI data during some years (Fig. 3) was the first indication that Georges Bank sea scallops spawn semiannually. Qualitative histological observations and quantitative analysis of female gonadal tissues (i.e., oocyte diameters and GVs) both support the presence of gametes during the late-spring, early-summer period. Mature oocytes were initially observed in February with greater amounts present by March. Mature oocytes were also observed in evacuating ducts, indicative of spawning, from samples collected as early as June 1990 and May 1991. Finally, the presence of planktonic larval stages was further evidence that spawning took place on Georges Bank in the spring of 1991. Tremblay et al. (1994) have suggested significant larval exchange between three distinct scallop aggregations studied on Georges Bank, therefore the larvae observed in this study may not have originated from the northeast portion of the bank. Tremblay et al. (1994) also suggest that Georges Bank is self-seeding, thereby supporting the conclusion that the sampled larvae of this study were not introduced from populations located off the bank.

A breeding season will usually be followed by a latent, non-reproductive period before gametogenesis is reinitiated (Giese and Pearse 1974). This resting period, usually associated with winter, has been reported for sea scallop populations in the Gulf of Maine (Robinson et al. 1981) and off the coast of Newfoundland (Thompson 1977). Further, Grant and Cranford (1989) reported that despite supplementary feeding, post-spawn sea scallops held

at ambient temperature in the laboratory could not be winter conditioned to reproductive maturity during their two month study period (October to December). These studies have all based their conclusions on gonadal indices, interpreting the lowest values following the autumn spawn when the scallop maintains a minimum gonad weight for several months, as a resting period. While GSIs are considered a rapid and accurate means of assessing reproductive activity in *P. magellanicus* (Beninger 1987), they do not allow one to differentiate between developmental stages of gametes in gonads (Beninger 1987, MacDonald and Bourne 1987). Histological preparation of gonadal tissues, although time consuming and more expensive than GSIs, allowed gametes to be directly observed during all phases of the reproductive cycle. Beninger (1987) and MacDonald and Thompson (1986), using histological analysis (i.e., oocyte diameter and GVs), observed that gametogenesis was reinitiated almost immediately following the autumn spawn. This agrees with the present study which observed no resting period following the major autumn spawn according to histological (i.e., microscopic) observations made in 1990, although GSI data (Fig. 3) seems to suggest a resting period for this, as well as other years. Even though corresponding GSIs indicated minimal gonadal weights, histological observations revealed the differentiation of new gametes as early as October (immediately after the autumn spawn) (Fig. 10A). Schmitzer et al. (1991) observed continual development of gametes between the spring and autumn spawns reported for mid-Atlantic Bight scallop populations. This was consistent with the Georges Bank population which revealed no summer resting period between observed semi-annual spawns. In fact, a spawn sampled off the coast of Delaware-Maryland was characterized by a dribble spawn during this interim period (Schmitzer et al. 1991).

DuPaul et al. (1989), Schmitzer et al. (1991), and Kirkley and DuPaul (1991) showed that, for southerly sea scallop populations, the spring spawn was larger and more consistent between years than the subsequent autumn spawn, which was not observed at all in 1989. This differs from Georges Bank, where autumn was the dominant spawning period (Fig. 3). Scallops sampled in the present study subsequent to the spring and prior to the autumn spawning periods were characterized by considerably heavier gonads than the minimal weights of spent gonads observed following the autumn spawn. Histological observations (oocyte diameter and GVF data) made between spawns revealed gonads occupied predominantly by developing gametes (oocytes and spermatocytes). This suggests that while the autumn spawn characteristically resulted in the evacuation of almost all of the resident gametes, only that fraction of gametes which reached maturity were actually released during the appropriate spring period (the spring spawn of 1991 was a partial spawn). This is supported by GVF data which revealed scallop gonads to have a considerably lower content of mature oocytes (i.e., gametes) in spring than in the autumn. Gonads sampled in the autumn consisted predominantly of mature gametes (Fig. 7A,B).

Semiannual reproductive cycles have been described for several other Pectinid species, including: *Pecten maximus* (Mason 1957, Gibson 1956, Comely 1974), *Pecten alba* (Sause et al. 1987), *Argopecten irradians* (Bricelj et al. 1987), *Chlamys distorta* (Reddiah 1962), and *Chlamys varia* (Reddiah 1962, Conan and Shafee 1978). Conan and Shafee (1978) have suggested, as one interpretation, that two distinct groups of individuals, each specialized for either a spring or autumn spawn, were present in the Bay of Brest. Observations of gonadal tissues sampled during

the spring and autumn spawning seasons of the present study revealed that all individuals possessed well-developed gametes with potentially viable and mature oocytes or spermatozoa. This suggests that all individuals prepared for and participated in both spawns.

The present study indicates that the extent of a semiannual reproductive cycle in *P. magellanicus*, as described by Schmitzer et al. (1991), must be extended northward to include the north-eastern portion of Georges Bank. Also, a semiannual reproductive cycle seems to occur more consistently in more southerly locations (mid-Atlantic Bight and Georges Bank). More northern populations, such as the Bay of Fundy (Beninger 1987, Parsons et al. 1992 and Newfoundland (Naidu 1970), have rarely if ever shown signs of spring spawning.

#### Resorbing Oocytes

Recent studies employing histological techniques (Barber et al. 1988, Schmitzer et al. 1991) have highlighted the importance of resorbed or atretic oocytes. These findings have important implications regarding reproductive strategies. The resorption of residual germ cells has been described in several other bivalve species (Loosanoff 1937, 1942, Bargerton 1943, Coe 1942, Cleland 1947, Roughley 1963). Mature, non-fertilized gametes are thought to remain viable within the gonad for a limited period of time, with those not spawned becoming atretic (Pipe 1987, Dorange and LePenne 1989). More recent studies have suggested that the products of gamete atresia may be resorbed and the energy reallocated to still-developing oocytes or used for other metabolic purposes by the bivalve (Herlin-Houtteville and Lubet 1975, Lubet et al. 1987, Dorange and LePenne 1989, Motavkine and Varaksine 1989). Therefore, the continual production and resorption of successive gamete cohorts may actually be viewed as an adaptation to environmental constraints (e.g., temperature, food availability; Morvan and Ansell 1988, Paulet 1990).

Temperate species, such as *P. magellanicus*, usually are exposed to a phytoplankton bloom in early spring. This translates into a limited period of time to build energy reserves (Thompson 1977, Robinson et al. 1981, Thompson and MacDonald 1990) while meeting immediate energetic demands through the direct uptake of food. A species adapted to initiate gametogenesis at this time might be expected to maximize reproductive investment. However, the fate of this initial investment will depend on environmental conditions experienced following the bloom. For example, in a case where the initial reproductive investment (energy allocated to the production of gametes) was too large, nutritive reserves may not be extensive enough to allow all eggs to reach the critical size for spawning. It might be expected that the bivalve should possess a mechanism to resorb and utilize the high nutritive content rather than releasing non-viable gametes. Oocyte lysis was a common observation in Georges Bank sea scallops possessing mature oocytes.

MacDonald and Thompson (1985b) suggested that, in an environment such as their Newfoundland study site, an appropriate strategy for *P. magellanicus* may be to invest surplus energy into the production of gametes. The individual scallop, therefore, will maximize reproduction according to the availability of resources; the better the location (e.g., greater food supply) the larger the reproductive investment. As previously noted, Langton et al. (1987) have suggested that sea scallops will "switch" annually from a synchronous to a protracted spawn depending on environ-

mental conditions. Therefore, variations in exogenous factors (e.g., water temperature and food availability) may produce considerable variation in the nature of semiannual or annual spawns (synchronized, protracted, or dribble). Georges Bank GSI data (Fig. 3) illustrate this cycle very well with two distinct spawns (spring and autumn) in 1986, a dribble spring spawn in 1988, and no spring spawn in 1989.

#### Larval Sampling

This is the first report of scallop larvae sampled from north-eastern Georges Bank outside of the autumn spawn. A program monitoring larval abundance approximately 1.8 km east of Hampton Beach, NH (42°54'N, 70°48'W) has also identified sea scallop larvae in late spring from 1977 to 1980 (Savage 1980). The range in age of sampled larvae in this study (i.e., estimated by size according to Culliney (1974) and Tremblay, pers. comm.) suggests a difference of about 3 to 4 weeks between the youngest (smallest) and oldest (largest) larvae. However, colder water temperatures at this time of year may make this an underestimate of age. Finally, it is unlikely that those larvae sampled could have overwintered from the previous year's autumn spawn.

#### Implications for a Commercial Fishery

The significance of a spring spawn to recruitment processes for the Georges Bank scallop population has not been established. However, a semiannual spawn could have important implications for presently employed sea scallop population analytical models. Georges Bank scallop stock assessment uses cohort analysis (i.e., sequential population analysis; Caddy 1989, Robert and Black 1990) to analyze historical fishery data (catch and effort data) and make estimates of population parameters (numbers-at-age, biomass-at-age and F-at-age). Subsequently, a predictive analytical model based on yield per recruit is employed to make catch projections utilizing the same population parameters to determine different management strategies. Because these are age-structured models, it becomes apparent that errors in aging the stock could produce errors in population parameters and, subsequently, the final assessment. Although the effect of a semiannual reproductive strategy on stock assessment models is beyond the scope of this paper, a few points of interest are outlined below.

Juvenile scallops less than one year of age that are collected from northeastern Georges Bank have exhibited considerable differences in size, falling into two predominant shell height categories (~5 to 10 mm and 20 to 25 mm, Thouzeau pers. comm.). This is likely to be attributable to two separate spawns of scallops on Georges Bank. Thouzeau (pers. comm.) has also observed this distinction to persist in two-year-olds, when scallops may initially be caught by fishing gear employed on Georges Bank (Caddy 1989).

Mason (1957) and Conan and Shafee (1978) observed similar differences in shell-height measurements made on spring and autumn cohorts of *P. maximus* and *C. varia*, respectively. Both Mason (1957) and Conan and Shafee (1978) reported that these discrepancies in size between cohorts persist up to age 4 and 5 years, although statistical significance was not reported.

If a significant difference persists to an age of 4 and 5 years in sea scallops, the age at which they recruit to the fishery, and if no allowance is made for the difference in size of spring- and autumn-spawned individuals, then an age-related error will be introduced into population parameter estimates and yield-per-recruit models. For example, two cohorts of the same year, significantly different

in size, could be expected to reveal a preferential retention of spring spawned (i.e., larger) scallops by fishing gear. In such cases, we have no apparent way of determining the relative importance of these respective cohorts to year class strength and cannot apply direct changes to the methods of analyses, presently employed, to compensate for the suggested skewed estimates of determined age-structured parameters. Further, because 4- to 5-year old sea scallops from Georges Bank made approximately 50 and 38% of total catch between 1986 and 1989, respectively (Robert and Black 1990), this error could be significant. This would be especially true when a significant contribution to the annual recruitment pattern was made by the spring spawn.

In conclusion, the occurrence of spring spawning on Georges Bank could have important implications for age-based analytical models used to derive population estimates. However, reevaluating techniques will depend on more rigorous consideration of the respective spawning strategies. The relative importance of the spring and autumn spawns to future year class strength is of particular interest, as is the significance of interannual variations in their relative contribution.

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## DEVELOPMENT OF THE FISHERY FOR WEATHERVANE SCALLOPS, *PATINOPECTEN CAURINUS* (GOULD, 1850), IN ALASKA

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**ABSTRACT** The Alaska scallop fishery harvests weathervane scallops, *Patinopecten caurinus* (Gould 1850), in the Gulf of Alaska and Bering Sea, although small quantities of *Chlamys* spp. were harvested in recent years. The fishery began in 1967 and evolved from a sporadic, low-intensity fishery to one characterized by a highly specialized fleet by 1993. An influx of larger, more efficient vessels from 1990 through 1993 increased harvests and altered the character of the fishery. Vessel length increased 85% from a mean ( $\pm 1$  standard error) of  $18.5 \pm 2.9$  m in 1983 to  $34.3 \pm 4.5$  m in 1991, and crew sizes doubled. The number of scallop landings increased significantly from  $65.9 \pm 8.3$  y<sup>-1</sup> during 1980 through 1989 to  $140.7 \pm 3.3$  y<sup>-1</sup> during 1990 through 1993, although the mean number of vessels did not change significantly between the two periods. Scallop harvests averaged  $667.1 \pm 54.8$  mt of shucked meats from 1990 through 1993, three times the average harvest of  $216.7 \pm 30.3$  mt from 1983 through 1989. The percentage of the fleet's total Alaskan fishing income derived from the scallop fishery increased from 57.7% in 1983 to 100% by 1990. The decreased diversification of scallop vessels into other fisheries represented a shift from a part-time fleet to a dedicated, full-time scallop fleet with greater harvesting efficiency. New management measures were adopted to address the changing nature of the fishery and included altered fishing seasons, observer coverage, area harvest limits, ceilings on catch of incidental species, restrictions on crew size and a moratorium on vessels fishing in the exclusive economic zone.

**KEY WORDS:** Scallops, fishery, *Patinopecten caurinus*, Alaska

### INTRODUCTION

The commercial fishery in Alaska for weathervane scallops, *Patinopecten caurinus* (Gould 1850), is a relatively small fishery occurring primarily in the Gulf of Alaska and the Bering Sea in the northeastern Pacific Ocean. Although other scallop species (*Chlamys pseudoislandica* and *C. rubida*) were harvested in 1991 and 1992 in small directed fisheries in the Aleutian Islands or caught incidentally in the fishery for weathervane scallops, their contributions to the total harvest were minimal.

Interest in the Alaskan fishery developed in the late 1960s at the time catch-per-unit-effort (CPUE) was declining in the U.S. and Canadian scallop fisheries on Georges Bank (Orensanz 1986). The decreasing supply of Georges Bank scallops to U.S. markets and increased prices encouraged development of new fisheries and expansion of existing fisheries worldwide to meet the demand for scallops (Caddy and Lord 1971).

The contribution of the Alaskan fishery to the total U.S. harvest of scallops is small, but has grown in recent years. Landings for Alaska and U.S. are commonly reported as shucked meat weight (e.g., U.S. Dept. Commerce 1993). The Alaskan percentage of the U.S. harvest increased from an average of 1.7% from 1980 through 1985, to 2% from 1986 through 1990, to 4% in 1991 through 1992 (U.S. Dept. Commerce 1982-1993). The larger percentage resulted from declining harvest in other U.S. fisheries and increased harvest in the Alaskan fishery. Worldwide scallop landings are commonly reported as live whole weight (e.g., Anonymous 1991). Shucked weight of weathervane scallops averages 11.5% of whole weight (Haynes and Powell 1968). Thus, in 1991 the Alaskan harvest of 536 mt shucked weight was approximately 4,661 mt (0.57%) of the world scallop harvest of 816,000 mt whole weight (Anonymous 1991).

Because the Alaskan scallop fishery was relatively minor until recently, it was passively managed and data collection was minimal. Without a time series of routine abundance and catch sampling data, historical description of stock dynamics is not possible. Standardized CPUE data are unavailable. In any case, CPUE data may not be indicative of relative abundance due to the spatial distribution of scallop beds and the pattern of vessel movement among beds to sustain high CPUE (Orensanz et al. 1991). On the other hand, good records on landings, vessels, and participants have been maintained since the inception of the fishery.

In this paper, we review the geographic distribution of weathervane scallops in Alaska and chronicle the development and management of the commercial fishery, with particular emphasis on the evolution of the scallop fleet from a part-time, diversified, small-vessel fleet to a full-time, large-vessel fleet fishing almost exclusively for scallops.

### MATERIALS AND METHODS

We analyzed databases of commercial vessel license applications, commercial fishing permit applications, and fish tickets which are maintained by the Alaska Department of Fish and Game (ADF&G) and Commercial Fisheries Entry Commission. Vessel license applications contain data on fishing vessels such as length, whereas fishing permit applications include information on permit holders such as residency, age, fishery and type of fishing gear.

Fish tickets are sales receipts which include information such as the date of landing, species, number and weight of fish caught, and area of harvest. In Alaska, scallop landings data are generally considered to be accurate, because there have been fewer than 20 vessels to monitor and overall compliance with fish ticket requirements is excellent. The scallop harvests reported herein include all

species, and represent weights of shucked meats without roe. Alaska state statutes protect the confidentiality of individual fishers' harvests and earnings. To maintain the confidentiality of this information, we omitted scallop harvest and earnings data when the number of persons or number of vessels participating in the fishery was less than four. For this reason, data were considered confidential in 1976, 1977 and 1979.

### The Weathervane Scallop

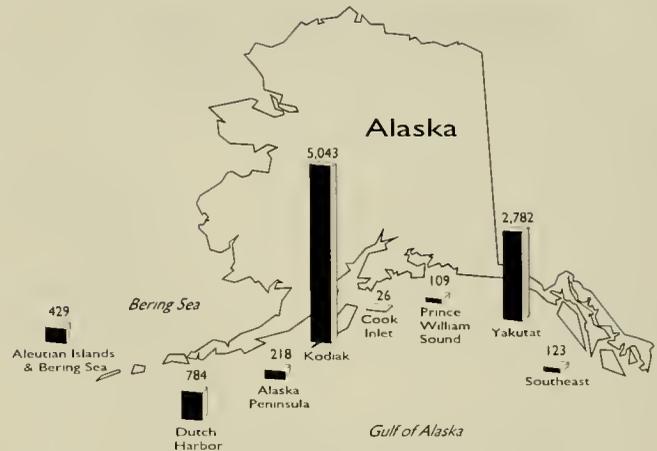
The weathervane scallop, *P. caurinus*, is a large, long-lived species. The largest specimen recorded in Alaska was 250 mm shell height (SH), and had an adductor meat weight of 340 g (Hennick 1973). Bourne (1991) reported weathervane scallops over 20 years of age from the Strait of Georgia, British Columbia, and scallops up to age 28 have been found in Alaska (Hennick 1973).

Weathervane scallops are distributed along the western coast of North America from California northward to the Bering Sea and westward as far as the Aleutian Islands in depths to 300 m (Grau 1959, Bernard 1983). Commercial quantities of weathervane scallops in Alaska are located primarily in the relatively shallow waters of the submerged continental shelf (Kaiser 1986, Fig. 1). From 1973 through 1976, 68 to 100% of the calculated scallop biomass in Alaska was reported at depths of 0 to 100 m, and commercially harvestable quantities were identified at depths of 46 to 128 m (Ronholt et al. 1977). *P. caurinus* in the northeastern Gulf of Alaska comprised 31% to 37% of the benthic biomass, at depths of 100 m or less (Feder et al. 1981). Unfortunately, scallop assessment surveys were conducted in only a few years and their geographic coverage was limited.

Locations of commercially important beds of *Placopecten mangellicus* on the Atlantic coast were characterized in areas with oceanographic features, including persistent tidal gyres, which contribute to retention of pelagic larvae in the area during the larval period (Sinclair et al. 1985, Caddy 1989). The dominant oceanographic feature of the Gulf of Alaska is a permanent, counterclockwise gyre of the Alaska Current which generally parallels the continental slope at velocities of 30 to 100 cm s<sup>-1</sup>. The velocities are relatively slow, but as the current narrows into the Alaskan Stream from Kodiak Island westward, velocities increase to a maximum of 100 cm s<sup>-1</sup> (Hood 1986). Shoreward of the shelf break, the Alaska Coastal Current borders the Gulf of Alaska from the southeastern panhandle to beyond Kodiak Island and into the Bering Sea. The Alaska Coastal Current flows counterclockwise and is driven by wind and freshwater discharge (Royer 1983).

The largest commercial scallop harvests in Alaska were produced from the coastal margin of the Gulf of Alaska near Kodiak and Yakutat (Fig. 1). Smaller harvests occurred in Southeast Alaska, Prince William Sound, Cook Inlet, Dutch Harbor and the Bering Sea. Analyses have not been completed to determine if scallops in these areas are genetically distinct populations, although larvae could conceivably be transported by currents between some of the closer areas.

Weathervane scallops are found in aggregations which tend to form elongated beds, oriented along the direction of current flow as described for other scallop species (Orensanz 1986, Caddy 1989), on substrates of sand, mud, clay and gravel (Hennick 1973). Growth and size at maturity of weathervane scallops may vary spatially within beds in the same area or between beds in geographically different areas (Haynes and Hitz 1971, Orensanz



**Figure 1.** Distribution of total commercial scallop harvest in the Gulf of Alaska and Bering Sea from 1967 through 1993. Cumulative harvests in metric tons of shucked meats are indicated above the bar for each area; bar height is proportional to the area harvest.

1986, MacDonald and Bourne 1987, Caddy 1989). Based on von Bertalanffy growth estimates, weathervane scallops sampled in 1975 from Marmot Flats off the northeast side of Kodiak Island grew faster (131 mm SH at age 4) and achieved larger asymptotic sizes ( $L_{\infty} = 190$  mm SH) than those sampled in 1980 from Cape St. Elias to Cape Fairweather in the eastern Gulf of Alaska (91 mm SH at age 4;  $L_{\infty} = 144$  mm SH) (Kaiser 1986). Growth and mortality rates may be density dependent (Orensanz 1986) or may vary with spatial differences in temperature or feeding conditions (MacDonald and Thompson 1985).

Annual survival of long-lived species, such as the weathervane scallop, tends to be high. Kruse (1994) estimated the instantaneous natural mortality ( $M$ ) of *P. caurinus* between 0.04 and 0.25, corresponding to annual mortality rates of 4 to 22%. A median estimate of  $M = 0.16$ , corresponding to 15% annual mortality, was obtained using Hoenig's (1983) method based on a Hennick's (1973) maximum scallop age of 28 (Kruse 1994).

The level of fishing mortality for weathervane scallops in Alaska is unknown. For other species of commercially important scallops, direct and indirect mortalities have been identified in association with fishing activities. Direct mortality includes damage or crushing within scallop dredges, and handling and exposure of undersized scallops that are later returned to the sea (Naidu 1988, Medcof and Bourne 1964). Indirect mortality results when scallops in the path of a dredge are not caught but are lethally damaged or exposed to increased predation as predators are attracted to dredge tracks (Caddy 1968, Elner and Jamieson 1979, Caddy 1973). Indirect fishing mortality of Iceland scallops, *Chlamys islandica*, was estimated to range up to 17% or 31%, depending on the type of dredge used (Naidu 1988).

The vulnerability of scallops to fishing mortality may be increased by commensal or parasitic organisms. Scallops in the Cape Yakutat/Cape Yakutat region of Alaska were reported to be infested by the burrowing, spionid polychaetes *Pygospio elegans* and *Polydora ciliata* in the mid- and late-1970s (Feder et al. 1981). The polychaetes weakened the valves and increased the susceptibility of scallops to breakage in trawls or dredges.

### The Fishery

The commercial scallop fishery progressed through several developmental phases along the Alaskan coast. In the initial phase

from 1967 through 1977, previously unexploited scallop beds were explored and harvested. Scallop landings were greatest in the years 1968 and 1969 (Fig. 2). Harvest effort decreased from 1970 to 1978, and a smaller, more stable fishery followed from 1979 through 1989. Beginning in 1990, the fishery for weathervane scallops in Alaska expanded with an influx of new, more efficient vessels. New state and federal management regimes were implemented in 1993 and 1994 to address concerns in the growing fishery.

The first commercial landings of weathervane scallops in the Alaskan fishery were made in 1967 in the western Gulf of Alaska near Kodiak Island. The fishery consisted of two vessels which delivered scallops alive and in the shell to processors on shore for an ex-vessel price of  $\$0.15 \text{ kg}^{-1}$  (Kaiser 1986). The fishery expanded rapidly in 1968 and 1969 when 19 vessels entered the fishery in the Kodiak area and the eastern Gulf of Alaska near Yakutat. A record harvest for the Alaskan fishery of 839.2 mt was produced in 1969. The costly process of shucking scallops by shore-based processors was soon abandoned, and scallopers began delivering shucked meats to processors for freezing and packaging (Kaiser 1986). The weighted average ex-vessel price for shucked meats in 1969 was  $\$1.89 \text{ kg}^{-1}$ . Unsuccessful attempts were made to extract scallop mantles and gonads for sale as frozen food (ADF&G 1968). To this date, a market for "roe-on" scallops has not developed, and shucked meats continue to be the only product of the Alaskan scallop industry.

During the years 1970 through 1978, the scallop fishery entered a phase of declining effort and harvest. Harvest levels of 1968 and 1969 were not sustained. More restrictive management measures, such as closed areas to limit bycatch, may have contributed to a reduction in the fishery. A catch sampling program conducted during 1968–1972 showed that the largest scallops were fished from previously unexploited populations. Hennick (1973) estimated catch age composition by enumerating annuli on the left valves of scallops. In the early years of the fishery the majority of scallops sampled from commercial harvests were at least 7 years old, but by the early 1970s, the percentage of scallops 2 to 6 years old had increased (Hennick 1973, Fig. 3). Scallopers continued to explore for new beds in attempts to boost their harvests in response to the decreasing abundance of large scallops.

The number of vessels in the fishery dropped from 19 in 1969 to 7 in 1970, but because the most efficient scallop vessels remained in the fishery (Kaiser 1986), the relatively high harvests continued from 1970 through 1973 and mean ( $\pm 1$  standard error

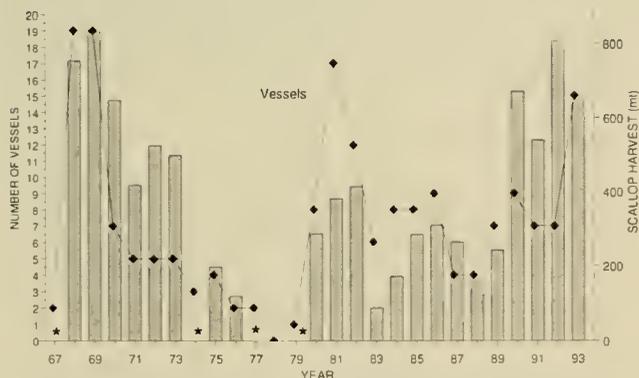


Figure 2. Annual scallop harvest (metric tons of shucked meats) and number of vessels fished in the Alaskan scallop fishery. Confidential harvests are indicated by an asterisk (\*).

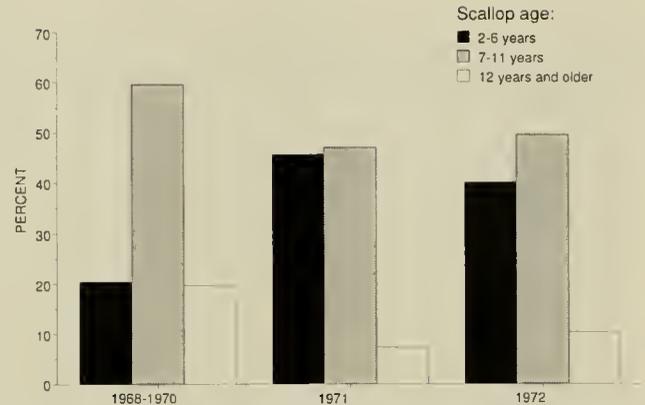


Figure 3. Age composition of scallops sampled from 1968 through 1972 commercial harvests from the Kodiak and Yakutat areas combined (Hennick 1973).

landings were  $522.9 \pm 94.9 \text{ mt y}^{-1}$ . By 1977, the number of vessels had dwindled to two. The fishery was opened in 1978, but no vessels participated and commercial harvest of scallops ceased.

The scallop fishery emerged again in 1979 with one vessel fishing off Kodiak Island. Marked increases in ex-vessel prices from an average of  $\$3.49 \text{ kg}^{-1}$  in 1976 and 1977 to  $\$6.11 \text{ kg}^{-1}$  in 1979 to  $\$9.23 \text{ kg}^{-1}$  in 1981 stimulated more interest in the fishery, and the number of vessels increased to 17 by 1981. Kaiser (1986) attributed much of the growth in the Alaskan fishery to an influx of vessels from the east coast of the U.S. A five-fold increase in the number of vessels in the Atlantic sea scallop fishery from 1975 through 1979 and a 22% decrease in the sea scallop harvest from 1978 through 1981 (NEFMC 1982) caused scallopers to move to the west coast in search of more productive scallop fisheries. This search led to an intense fishery for weathervane scallops off the Oregon coast in 1981 in which 532 mt (shucked weight) was harvested by 118 vessels (Starr and McCrae 1983). Subsequent landings in Oregon have been small (Bourne 1991).

Vessels began harvesting scallops from new areas of Alaska in the 1980s. Whereas previous fisheries occurred entirely in the Kodiak and Yakutat regions of the Gulf of Alaska, an increasing percentage of the harvest in the 1980s was from less traditional areas. Scallops were harvested from Southeast Alaska along the Alexander Archipelago south of Yakutat, for the first time in 1980. The first scallop harvest from the Alaska Peninsula in the western Gulf of Alaska south of Kodiak was recorded in 1982. Harvests were reported from the Dutch Harbor area of the Aleutian Islands beginning in 1982, and the first harvest from Cook Inlet was produced in 1983. Expansion of the scallop fishery continued into the Bering Sea in 1986. From 1983 through 1989, effort in the Alaskan fishery varied from 4 to 9 vessels. The harvest level fluctuated during that period, and averaged  $216.7 \pm 30.3 \text{ mt y}^{-1}$ .

Expansion of the fishery continued as scallopers explored and exploited new scallop grounds further west along the Aleutian Islands chain to Adak in 1991. In 1992, scallops were harvested from Prince William Sound in the north central Gulf of Alaska for the first time.

The period from 1990 through 1993 represented the most recent growth phase in the fishery. The average harvest more than tripled from an average of  $216.7 \pm 30.3 \text{ mt y}^{-1}$  during 1983 through 1989 to  $667.1 \pm 54.8 \text{ mt y}^{-1}$  from 1990 through 1993. The 1992 harvest of 806.9 mt, with an ex-vessel value of  $\$6.9$

million, was the second largest recorded in the Alaskan scallop fishery. Although scallop harvests were much larger, effort was similar to that of recent years. Nine vessels fished in 1990 and 7 vessels in 1991 and 1992.

The number of vessels in the scallop fishery doubled to 15 in 1993. As in the early years of the fishery, many of the new vessels entering the fishery from 1990 through 1993 were east coast scallopers. A proposed moratorium in the Atlantic sea scallop fishery (NEFMC 1993) caused some vessels, which would be excluded from the east coast fishery by the moratorium, to move into west coast scallop fisheries.

In 1993, the North Pacific Fishery Management Council (NPFMC) considered a vessel moratorium for the Alaska fishery within the exclusive economic zone (3–200 miles) and gave notice to scallopers that new participants in the fishery after January 20, 1993 may not qualify to fish during a moratorium (NPFMC 1993). Effort escalated in anticipation of a moratorium in the Alaska scallop fishery as vessel owners established their eligibility by harvesting scallops in Alaska prior to January 20, 1993. State management agencies also considered a moratorium on new entrants within territorial (0–3 miles) waters (Kruse et al. 1992).

#### Vessel Length

Vessel size in the Alaskan scallop fishery increased substantially from the onset of the fishery in 1967. During the developmental period of the Alaskan fishery, scallop vessels consisted of a variety of converted crab, halibut and shrimp vessels, as well as scallop vessels from the east coast of the U.S. The east coast vessels averaged 24 to 28 m keel length and were capable of fishing two scallop dredges 3 to 5 m wide. These were the most efficient harvesters in the fleet (Kaiser 1986). Other vessels were fishing modified beam and otter trawls and an assortment of scallop dredges of various sizes.

Kaiser (1986) summarized vessel lengths in the scallop fishery from ADF&G data for 1967 through 1981. Vessels that fished in the Kodiak area were reported separately from vessels in Yakutat, although some vessels fished in both areas. Vessels that fished near Yakutat varied in length from 12 to 16 m to 24 to 28 m from 1967 through 1977. The largest number of vessels each year is 24 to 28 m in length. Similarly, most scallop vessels that fished in the Kodiak area were 24 to 28 m, although the size range for Kodiak vessels was greater than the range for Yakutat vessels in the early years of the fishery. Kodiak vessels varied from a 6 to 10 m length class to 48 to 52 m in length.

Vessel lengths after 1977 are shown in Figure 4 for all fishing areas combined. The largest component of the fleet in most years was the 18 to 31 m vessel class (Fig. 4A). Registered vessel length averaged  $25.1 \pm 0.8$  m from 1980 through 1982 (Fig. 4B). An influx of vessels smaller than 18 m was evident beginning in 1982 and was associated with initiation of a scallop fishery in the Cook Inlet area where vessels tended to be smaller than vessels fishing in other areas. The mean vessel length reached a minimum of  $18.5 \pm 2.9$  m in 1983 as a result of the increase in small vessels.

By 1988 all scallop vessels in the Alaskan fleet were larger than 18 m. Mean vessel length increased as the number of vessels over 31 m began to rise. Vessel size was largest during the years 1990 through 1992, when the percentage of vessels over 31 m varied from 30 to 57% of the fleet (Fig. 5). Mean length in 1991,  $34.3 \pm 4.5$  m, was 85% larger than the mean length of  $18.5 \pm 2.9$  m in 1983. The mean vessel length dropped to  $26.8 \pm 1.7$  m as more vessels under 31 m in length entered the fishery.

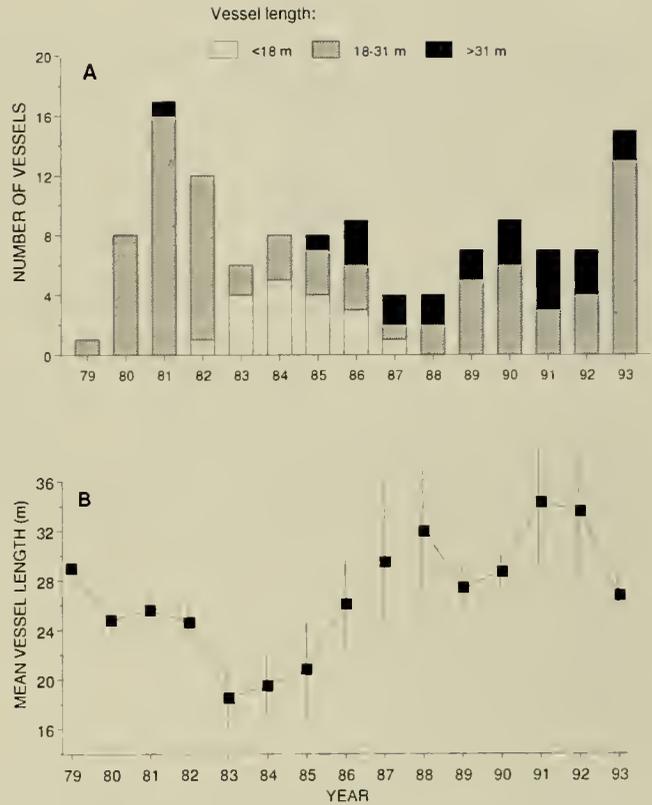


Figure 4. (A) Annual number of vessels in the Alaska scallop fishery from 1979 through 1993, by vessel length class. (B) Annual mean vessel lengths  $\pm 1$  standard error.

#### Fishing Capacity

One of the more important changes in the scallop fishery was the change in fishing capacity of the fleet. Fishing capacity was measured as the mean number of scallop landings (deliveries) per year and the mean size of landings.

In a previous study, we reported an escalation in the number of landings in the Alaskan scallop fishery in recent years (Kruse and Shirley 1994). A Student's *t*-test (SAS 1989) revealed that the mean number of landings of  $140.7 \pm 3.3$  in 1990 through 1993 was significantly larger ( $p \leq 0.01$ ) than the mean of  $65.9 \pm 8.3$  landings in 1980 through 1989. The number of landings increased although the mean number of vessels fished per year from 1990 through 1993,  $7.7 \pm 0.7$ , was not significantly different from the number of vessels fished in the earlier period,  $8.3 \pm 1.2$ . The mean landing size (in mt) was not significantly different between the two time periods.

#### Diversification of Fishing Income

The proportion of Alaska fishing income derived from the scallop fishery was used as an indicator of the economic dependence of vessels on the scallop fishery and the extent of effort exerted in the fishery by these vessels. All ex-vessel earnings (gross receipts paid to fishers) from Alaskan fisheries were compiled annually for vessels that made scallop landings each year. Scallop earnings were calculated as a percentage of the scallop fleet's total earnings to estimate the degree of diversification from 1975 through 1992. The fleet was considered to be more diversified in years when the percentage of fishing income derived from scallops was low and



Figure 5. The commercial scallop vessel F/V PROVIDER which is an example of the type of vessel which has been used in the Alaskan scallop fishery since 1990. (Photo by Augustine Delahay)

less diversified in years when scallops contributed larger percentages to the fishing income.

The percentage of fishing income derived from the scallop fishery varied over time from a low of 57.7% in 1983 to 100% in 1979, 1990 and 1991 (Fig. 6). In 1975 through 1979, effort and harvests were reduced, and almost all fishing income for the few vessels during that period was generated from the scallop fishery.

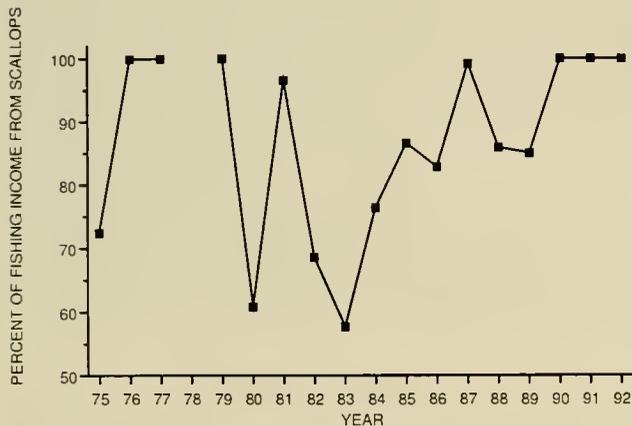


Figure 6. Percent of total ex-vessel fishing income derived from the scallop fishery for vessels participating in the fishery from 1975 through 1992. Total fishing income does not include any income generated from fisheries outside of Alaska.

Small percentages of non-scallop income were produced from king crab, halibut and salmon fisheries.

The contribution of scallops to total fishing income generally decreased after 1979 to the lowest level in 1983, suggesting an increased diversification of the fleet into fisheries other than scallops. Scallop vessels were also used in king crab, Tanner crab, shrimp, salmon, halibut and herring fisheries during that period.

Diversification of the scallop fleet began to change in 1983. The percentage of fishing income produced from the scallop fishery increased from 1983 to 1990. By 1990, scallops contributed 100% of the fishing income for the first time since 1979. All of the fishing income for the scallop vessels from 1990 through 1992 was produced from the scallop fishery. The change in fishing income represented a shift from a diversified fleet in the 1980s to a fleet concentrating almost entirely on scallops. The number of landings and the total harvest increased concurrently.

The contribution of the scallop fishery to total fishing income was not directly related to ex-vessel price of scallops or to the total scallop harvest. Ex-vessel prices remained relatively stable from 1982 through 1992, although when corrected for inflation, the price of scallops decreased over time (Kruse and Shirley 1994).

#### Harvesting Efficiency

The harvesting efficiency of the scallop fleet improved during the 1980s and 1990s. As vessels became larger and more specialized, crew sizes expanded and mechanical shuckers came into use. The average crew size for scallop vessels during 1980 through

1984 was estimated to be 5 persons per-vessel in the Yakutat area, 6 per-vessel in Prince William Sound, 6.3 per-vessel in Cook Inlet, the Alaska Peninsula, Aleutian Islands and Bristol Bay, and 8 per-vessel in Kodiak (Alaska Department of Labor, unpublished). By 1993, all vessels except the smallest in the fleet carried 12-person crews (NPFMC 1993). The Alaska Board of Fisheries limited the crew size on scallop vessels to 12 persons in 1993.

The use of mechanical shucking devices in Alaskan scallop fisheries was first reported in the 1991 fishery (Griffin and Ward 1992). Automatic shucking machines were employed to make harvest of smaller scallops more economical (Kruse and Shirley, in press). Although shucking machines were used primarily on vessels exploiting *Chlamys* spp., their use in processing weathervane scallops was banned in Alaska in 1993.

## DISCUSSION

Alaska's commercial fishery for scallops evolved from a sporadic, low-intensity fishery to a fishery characterized by a highly specialized fleet capable of harvesting with greater efficiency. This growth and specialization within the fishery created conservation concerns for the scallop resource. Recent anecdotal reports indicated that the proportion of smaller scallops harvested in the commercial fishery may be increasing (NPFMC 1993). Additional concern has arisen over the increased threat to species caught incidentally in non-selective scallop dredges. Particularly vulnerable are benthic migratory species, such as crab, which move into shallower depths during their molting and mating periods (Haynes and Powell 1968, Hennick 1973, Stone et al. 1992). Protection of non-targeted species is especially critical in some areas of Alaska where depressed populations of commercially valuable king and Tanner crabs are rebuilding.

The rapid growth, specialization and over-capitalization of the scallop fishery may have jeopardized the economic viability of the fishery. Larger vessels and crews and mandatory observer coverage have increased operating costs for scallop vessels. Shorter fishing seasons and harvest limits have constrained the earning potential in the scallop fishery.

With increased competition, over-capitalization and decreased ex-vessel value, the tendency for some fisheries in Alaska has been for vessels to become more diversified by participating in more fisheries in order to maintain the level of income necessary to support their operations (Shirley 1993). More restrictive measures (e.g., harvest limits, seasons, crab bycatch caps) recently adopted by state and federal management agencies may also cause some scallop vessels to diversify their fishing activities.

Management changes have been made to address some of these problems in the fishery and to provide more scientific data to analyze the impact of the fishery on Alaskan scallop populations. For many years, the small scallop fishery prompted few conservation or management concerns, and was managed using gear restrictions, fishing seasons and closed areas (Kruse and Shirley, in press). The Alaska scallop fishery recently shifted from a passive management mode to more active management and monitoring of the fishery in response to expanded effort and increased harvest levels in the 1990s.

A management plan adopted in 1994 established a more comprehensive management regime for the scallop fishery (Kruse 1994). Scallop gear in Alaska was restricted to two New England-style dredges (Bourne 1964) per vessel, with a maximum dredge

width of 4.57 m and a minimum ring size of 10.16 cm. Dredges used in Cook Inlet cannot exceed 1.83 m in width. Alaskan waters were divided into 9 registration areas, and vessels are now required to register with ADF&G before harvesting scallops in an area. Annual harvest limits were established for each registration area. All scallop vessels are required to have, and pay for, observers on board to monitor incidental catch of crab and collect biological data on scallops. Restrictions on scallop crew size and prohibition of automatic shucking machines on vessels fishing for weathervane scallops were implemented to control harvesting efficiency. Fishing seasons were amended in the new plan to protect molting and mating crabs from incidental harvest by scallop dredges. The regulatory season extends from July 1 in most areas (August 15 in Cook Inlet) through February 15. In the Yakutat and Prince William Sound areas, the season opens on January 10. The fishing season ends when the harvest limit has been met, when the incidental catch limit has been met, or on the regulatory closing date, whichever comes first.

The NPFMC adopted a federal management plan for scallops in 1994 which included a moratorium on vessels fishing in the exclusive economic zone (NPFMC 1993). Vessels fishing scallops outside of territorial waters were previously managed by ADF&G in the absence of a federal management plan. Management changes provided by the new ADF&G and NPFMC management plans should help to curtail rapid expansion of the scallop fishery.

Because the weathervane scallop is a long-lived, slow-growing species with low natural mortality, it may be vulnerable to overfishing (Adams 1980). Historically, an overall depletion of scallop populations may have been avoided because of the widespread distribution of scallops in the Gulf of Alaska and the Bering Sea and the small fleet's motivation to move to new areas to maintain catch rates or to other fisheries. However, some local populations may have been overfished. As a fishery begins on virgin stocks it is normal for age composition to shift toward younger ages. However, the magnitude of the age shift during the early years of the fishery, coupled to subsequent fishery performance, suggests to us that high harvests during the early years of the fishery off Kodiak and Yakutat may not have been sustainable over the long term. Unfortunately, fishery-independent data are unavailable to verify whether overfishing occurred.

Oceanographic features similar to those described by Sinclair et al. (1985) and Caddy (1989) for self-sustaining scallop populations also may have contributed to the apparent resiliency of weathervane scallop populations in Alaska. Nonetheless, recent expansion of fishing capacity by the fleet concerned fishery managers who sought to conserve and sustain natural populations, and thereby avoid stock collapses that have often occurred in many other scallop fisheries after periods of intense fishing (Orensanz 1986, Bourne 1991, Gwyther et al. 1991, Orensanz et al. 1991, Piquimil et al. 1991). As biological data are collected from the new observer program, our knowledge of the sustainability of exploited weathervane scallop populations in Alaska should improve markedly with the estimation of recruitment, growth and mortality parameters.

## ACKNOWLEDGMENTS

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## MITOCHONDRIAL DNA VARIATION IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (LAMARCK, 1819), AND THE ATLANTIC CALICO SCALLOP, *ARGOPECTEN GIBBUS* (LINNAEUS, 1758)

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**ABSTRACT** Restriction site variation of the mitochondrial DNA of *Argopecten irradians* ssp. was surveyed within four populations from the U.S. Atlantic coast and the Gulf of Mexico. A population from North Carolina was resampled one year after the first collection to provide a measure of temporal variation within a population. Haplotype diversity was high, with 49 haplotypes revealed among a total of 135 bay scallops screened with 8 restriction endonucleases. Nucleotide sequence divergences corrected for within-sample variation among populations ranged from 0.00%, between the temporal samples, to 0.33% between geographically distant populations. Tests for heterogeneity indicated that no two of the geographically separated populations shared a common gene pool. UPGMA cluster analysis based on nucleotide sequence divergences suggested that bay scallops in North Carolina waters are more closely related to those from New England than to those from the Florida Gulf, though only slightly.

Two populations of the calico scallop, *Argopecten gibbus* Dall, from the Atlantic and Gulf coasts of Florida, were sampled and analyzed similarly. In these, haplotype diversity was high, with 6 restriction endonucleases revealing 19 different haplotypes among 51 individuals. Nucleotide sequence divergence between the geographically distant populations was low, however, and a test for heterogeneity was consistent with the null hypothesis of a shared, common gene pool for the two populations. No restriction fragment patterns were common to the bay and calico scallop, reinforcing their classification as separate species.

**KEY WORDS:** Bay scallop, calico scallop, *Argopecten*, mtDNA variation, geographic variation, restriction fragment length polymorphism

### INTRODUCTION

The genus *Argopecten* includes two species found in the Atlantic and Gulf coasts of the United States. *Argopecten irradians* Lamarck, the bay scallop, is a short-lived hermaphroditic species which grows rapidly and reaches reproductive maturity at a relatively young age. The calico scallop, *Argopecten gibbus* Dall, exhibits a life-history very similar to that of *A. irradians*, but dwells in a sandy-bottom offshore habitat rather than the shallow grass-bed regime of the bay scallop. Both species support economically significant fisheries (Shumway and Castagna 1994).

The bay scallop is distributed discontinuously along the Atlantic and Gulf coasts of the United States, between Cape Cod, MA and the Laguna Madre, TX. Frequently associated with eelgrass beds (*Zostera marina*), bay scallop populations are limited to estuarine and near-shore environments, with protected bays and sounds forming the primary habitat (Heffernan et al. 1988). Within its range, *A. irradians* has traditionally been divided into three subspecies whose geographic boundaries are not clearly delineated: the northern *A. i. irradians*, found from Cape Cod south to an area between New Jersey and Maryland; *A. i. concentricus*, found between New Jersey and North Carolina and on the Florida Gulf coast; and *A. i. amplicostatus*, whose range in the Gulf of Mexico extends south from Matagorda, TX and through the Laguna Madre (Clarke, 1965). In 1987, the existence of a fourth *A. irradians* subspecies, *A. i. taylorae*, was proposed by Petuch (1987). The holotype was collected in the Rabbit Key Basin of Florida Bay, and was distinguished from *A. i. concentricus* by its smaller and more fragile valves. The range of this subspecies is thought to be restricted to the dense, well developed *Thalassia* beds of Florida Bay and the waters west of the middle and upper Keys (Petuch 1987). The validity of this additional subspecies designation is complicated by the observation by Waller (1969)

that there exist numerous museum samples of *A. i. concentricus* reportedly collected from the Florida Keys.

It has been suggested that populations designated *A. i. concentricus* in North Carolina are more closely related to those of the northern bay scallop subspecies than to the Gulf populations with the same taxonomic designation, and the unique subspecific designation for the Rabbit Key scallops has been questioned (Marelli et al., in press). The degree of intergradation between the four currently recognized subspecies, and the extent to which they are genetically distinct, are not known.

Whereas the range of the bay scallop spans most of the eastern coast of the U.S., that of the calico scallop, *A. gibbus*, is limited to more southern waters. The calico scallop is found on the Atlantic coast between Cape Hatteras, NC and Cape Canaveral, FL. It is also present in harvestable numbers on the northern Gulf coast of Florida, and has been reported to occur off of the West Indies (Sastry 1962). Although their different habitats may effectively isolate the two species, the range of *A. gibbus* overlaps considerably with that of the southern bay scallop subspecies, *A. i. concentricus*, most notably off Cape Hatteras and in the Gulf of Mexico. *A. gibbus* does differ morphologically from *A. i. concentricus*, but the high degree of variation and overlap in the distinguishing characters can complicate identification (Sastry 1962).

The recruitment dynamics of bay and calico scallops are not fully understood, and it is likely that different strategies apply to the two species. For both, the range of larval movement or transport that may occur during the approximately two-week pelagic stage can be quite extensive. Kirby-Smith (1970) suggested that juvenile calico scallops found off of the North Carolina coast may be recruited from populations spawning on the Atlantic coast of Florida. The basis for this suggestion was the observation that calico scallop juvenile abundances off of North Carolina are not

correlated with the spawning of adults in the same, highly transient populations. Hydrographic conditions may permit the sporadic recruitment of larvae generated in Florida spawnings to beds off of North Carolina, perhaps through entrainment in the Gulf Stream (Kirby-Smith 1970). This hypothesis is supported by the low levels of morphological and allozyme variation detected between calico scallop populations from the Marquesas Keys, FL, Cape Canaveral, FL and Cape Lookout, NC (Krause et al. 1994). This type of long distance dispersal is not likely to occur for the bay scallop, whose habitat is restricted to near-shore grass beds and the shelter of barrier islands. It is thought that most bay scallop larval recruitment depends on tidal circulation which acts to retain the planktonic larvae within the suitable habitat of a bay or estuary (Marshall 1960, Peterson and Summerson 1992). If the different recruitment strategies of bay and calico scallops are indeed as hypothesized, one might expect to see a fairly continuous pattern of genetic variation along the geographic range of the calico scallop, whereas the more geographically isolated bay scallop populations should show higher levels of differentiation.

In addition to the large body of ecological work with scallop species, extensive genetic studies have been conducted, using techniques of chromosomal analysis and manipulation, and protein electrophoresis (Beaumont and Zouros 1990). Analyses of scallop population structure and stock isolation have used allozyme data primarily, as summarized by Beaumont and Zouros (1990), and Beaumont (1991). Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA has been widely applied in the field of marine population genetics (Ovenden, 1990), and the mitochondrial genomes of several members of the scallop family, Pectinidae, have been examined in some detail (Snyder et al. 1987, Gjetvaj et al. 1992). The family shows a broad range of mtDNA features, many very different from those of other metazoans. *Placopecten magellanicus*, the deep-sea scallop, exhibits the most dramatic divergence from the standard, with the largest known mtDNA molecule of any multicellular animal and a high degree of mtDNA size polymorphism within the species, attributed to varying copy numbers of a tandemly repeated sequence (Gjetvaj et al. 1992). No such sequences have been detected in the mtDNA of *A. irradians*, which has been found to possess a 16.2 kb molecule of invariant size. The mtDNA of the calico scallop, *A. gibbus*, has not been characterized by these techniques.

In this study we employed RFLP analysis of bay and calico scallop mtDNA to assess the levels of variation within and between populations of the same species, and between the two species. Specifically, we sought to evaluate the following null hypotheses: (1) geographically isolated populations of *A. irradians* share a common gene pool, (2) morphologically similar populations of *A. irradians* (i.e., those with the same subspecies designation) share a common gene pool, (3) mtDNA variation in a population of *A. irradians* is temporally stable, (4) individuals of the putative *A. i. taylorae* subspecies share a common gene pool with those of a sampled population of *A. i. concentricus*, (5) geographically isolated populations of *A. gibbus* share a common gene pool, and (6) *A. irradians* and *A. gibbus* share a common gene pool.

#### MATERIALS AND METHODS

Samples of 21 to 34 *A. irradians* were obtained from New England, North Carolina, Florida Bay, and the Florida Gulf coast (Table 1). These were assumed to approximate natural set in their

TABLE 1.  
Bay and calico scallop sample descriptions.

Population	Code	Date of Collection	n
Bay Scallops			
Crystal River, FL	FL	9/92	27
New England	MA	9/92	26
Harker's Island, NC	NC	10/12/92	27
Harker's Island, NC	NCT	11/12/93	21
Rabbit Key, FL	RK	9/22/93	34
Calico Scallops			
Cape Canaveral, FL	CA	2/17/93	27
Apalachicola, FL	AP	4/14/93	24

sites of origin. The New England sample was hatchery-produced with broodstock from Martha's Vineyard, Buzzards Bay and Nantucket Sound. The broodstock had been maintained in the hatchery since 1988, and was used to produce seed scallops to augment the natural bay scallop set in the area. The sample was harvested from seeded beds in Nascatucket Bay, but may have also contained scallops from natural set. The hatchery had not introduced any scallops from outside New England into their broodstock at the time of collection (R. Taylor, Taylor Seafood, 1993, pers. comm.). The sample from the Florida Gulf coast was composed of laboratory-reared F<sub>1</sub> progeny of individuals harvested from Crystal River, FL. The broodstock from Crystal River had not been combined with any other stocks (N. Blake, University of South Florida, 1992, pers. comm.). North Carolina bay scallops were collected from natural set on the clam beds of Carolina Cultured Shellfish, Inc., off of Harker's Island, North Carolina. A temporal replicate of this sample was obtained one year after the first collection. Scallops from Florida Bay were collected from the fairly pristine, though sparsely populated natural beds off Rabbit Key.

Samples of *A. gibbus* comprising 27 and 24 individuals respectively, were obtained from the waters off Cape Canaveral, FL and Apalachicola, FL (Table 1). They represent natural set for these regions.

With the exception of the Rabbit Key bay scallops and the Apalachicola calico scallops, samples were delivered or hand-carried to the Virginia Institute of Marine Science (VIMS) alive, where gonad and/or mantle tissue were dissected and frozen at  $-70^{\circ}\text{C}$ . Limited numbers of individuals were processed fresh when time permitted. In the exceptional cases, animals were shucked and frozen whole in liquid nitrogen, then stored at  $-70^{\circ}\text{C}$  until transport to VIMS on dry ice.

Mitochondrial DNA was purified by cesium-chloride density-gradient ultracentrifugation from 2–3 g of gonad or mantle and gill tissue, using a modified homogenization buffer containing 200 mM sucrose (Lansman et al. 1981). MtDNA yield was observed to vary with the type of tissue used, with ripe gonad providing better yield than mantle and gill, or immature or spent gonad.

Digestion of 10–50 ng of *A. irradians* mtDNA was carried out with the following restriction endonucleases according to the manufacturer's recommendations: *AvaI*, *BanI*, *BanII*, *BglII*, *BstEII*, *EcoRI*, *HaeII*, *HindII*. Many of the individuals in the *A. gibbus* sample from Apalachicola yielded low quantities of closed-circular mtDNA, perhaps because of an extended storage time, or inadequate handling procedures. Limited DNA quantities required that only six of the above enzymes be included in the calico scallop

analysis. These were *AvaI*, *BanI*, *BglII*, *BstEII*, *EcoRI*, and *HaeII*. For comparison with *A. irradians*, Cape Canaveral calico scallop DNA was screened with all eight enzymes.

DNA from all *A. irradians* samples and the Cape Canaveral *A. gibbus* were visualized by endlabelling. Restriction fragments were endlabeled with the Klenow fragment of DNA polymerase I and <sup>35</sup>S-labeled nucleotides, electrophoresed at 1 volt/cm in 1% agarose gels overnight, and visualized by autoradiography (Sambrook et al. 1989). <sup>35</sup>S-labeled 1 kb ladder DNA (BRL) provided a molecular weight size standard.

The low yields of mtDNA from Apalachicola calico scallops made it necessary to visualize some restriction patterns by Southern transfer and hybridization (Sambrook et al. 1989). Digests of mtDNA or aliquots of mtDNA-enriched nuclear DNA were prepared as described previously, and were electrophoresed in 1% agarose gels at 3 volts/cm for 3–4 hours, with biotinylated λ/HindIII fragments as a size standard. Gels were blotted by overnight capillary transfer onto nylon membrane, and membranes were UV-crosslinked to immobilize the DNA fragments. Blots were prehybridized for 2 hr at 42°C (50% formamide, 5× SSC, 5× Denhardt's solution, 0.025 mM NaPO<sub>4</sub>, pH 6.5, and 100 µg/mL heat-denatured calf thymus DNA). Overnight (42°C) hybridization was initiated with the addition of 0.25–0.5 µg biotinylated probe DNA prepared from purified *A. irradians*, *A. gibbus* or *Placopecten magellanicus* mtDNA by nick translation with biotin-7-dATP (BRL Bionick Labeling System). Following a series of post-hybridization washes (Sambrook et al. 1989) and blocking (3% bovine serum albumen), mtDNA fragments were visualized using the BRL BluGene Nonradioactive Nucleic Acid Detection System.

Sizes of mtDNA fragments were estimated by fitting band migration distances to those of the standard by the local reciprocal method of Elder and Southern (1983) using the program Gel Frag Sizer (Gilbert 1989). Restriction sites were inferred from completely additive fragment patterns, and letter designations were assigned to the different patterns. Six- or 8-letter composite haplotypes were compiled for the series of enzymes and analyzed separately for fragment and site data.

Statistical analyses were performed using the Restriction Enzyme Analysis Package (REAP) (McElroy et al. 1991). *A. irradians* and *A. gibbus* were analyzed separately, because they showed no similar restriction fragment patterns. For each sample, haplotype and nucleotide diversities were calculated following the methods of Nei (1987) and Nei and Miller (1990), respectively. Mean nucleotide sequence divergence between samples was calculated following Nei and Miller (1990), and was corrected for within-population polymorphism by subtracting the average of within-sample diversities. Since many of the haplotypes observed were rare, a Monte Carlo simulation (Roff and Bentzen 1989) was performed to estimate heterogeneity and assess the likelihood that the sampled populations shared a common gene pool.

RESULTS

*A. irradians*

Substantial genetic variation was observed within and among populations of the bay scallop. All enzymes revealed polymorphic restriction sites, producing between 4 and 10 distinct fragment patterns each. Analysis of 135 bay scallops with 8 restriction endonucleases revealed a total of 133 fragments and 49 distinct mtDNA haplotypes (Table 2). Individual enzymes revealed 6 to 16

TABLE 2.

*Argopecten irradians*. Composite haplotypes from seven populations.

Haplotype	MA	NC	NCT	FL	RK	Total
AAAAAAAAE	2					2
AABAAAAE	5					5
AABAAAAH	2					2
AACAAAAA	7	13	13	2		35
AACAAAAG	2					2
AACBAAAA	1					1
AADAAAAE	4					4
AAEAAAAE	2					2
AAFAAAAE	1					1
AACAAAAC		1				1
AACAAAAD		1				1
AACAAAAG		1				1
AACAABDD		1				1
AACABAAA		1				1
AACCAAAA		1				1
AAGAAAAE		1				1
ABCAAABA		1				1
ACCADAAA		1				1
ADCAAACA		1				1
AFCAAAAA		1				1
BACAAAAB		3		10		13
AEBAAAAA				2	3	5
AEBBAAAA				10		10
CABACAAA				2		2
AACAAAAI				1		1
AACAAAAE			1			1
AACAADAA			1			1
AACAEEAA			1			1
AACDAAAA			1			1
ACEAAAAE			1			1
AICAAAAA			1			1
DAIAAAAA			1			1
EACAAAAA			1			1
AABAAAAA					9	9
AABAAAAD					1	1
AABABA AJ					1	1
AABAEAAA					1	1
ACBAAAAA					1	1
ADBAAACA					1	1
AEBAAAAE					1	1
AEBAAAAH					1	1
AEBAAAEA					4	4
AEBAAAEK					1	1
AEBDAAAA					3	3
AGCAAAAA					1	1
AHHABAHA					1	1
DABABCAA					1	1
DCBABA AK					3	3
EEBDAAAA					1	1
Total n	26	27	21	27	34	135

MA = New England; NC = Harker's Island, NC; NCT = NC + 1 yr.; FL = Crystal River, FL; RK = Rabbit Key, FL. Restriction enzymes used: *AvaI*, *BanI*, *BanII*, *BglII*, *BstEII*, *EcoRI*, *HaeII*, and *HindII*.

restriction sites. Eighty-three sites were scored in total, and restriction site gains and losses were inferred from additive changes in fragment patterns. The most common composite haplotype, represented by 35 individuals, contained a total of 52 sites, accounting for approximately 1.7% of the mtDNA genome. The size

of the whole molecule was estimated at 16.7 kb from *AvaI* digests, and no indications of size polymorphism or heteroplasmy were apparent from these RFLP data.

Haplotype diversity, or the probability of encountering different haplotypes when two individuals are sampled from a population, was quite high (0.63–0.91) for all of the bay scallop samples (Table 3). Mean nucleotide sequence diversities ranged from 0.22%, for the second North Carolina sample (NCT), to a high of 0.53% for the Crystal River sample (FL). The high value for the latter population was not correlated with haplotype diversity, but was caused by the predominance of two divergent haplotypes in the sample, AEBBAAAA and BACAAAAB, that differed from each other by 5 sites. The first North Carolina (NC) sample contained 11 rare haplotypes, each represented by a single individual, but 9 of these differed from the common haplotype by only one or two site changes. Thus, the nucleotide sequence diversity within this population was comparatively low at 0.28%.

A matrix of mean nucleotide sequence divergences among the populations is presented in Table 4. Values below the diagonal are corrected for within-sample variation. Most notable among these is the corrected divergence between the two North Carolina samples (NC and NCT) collected at the same site in consecutive years. This value is negligible, indicating that the likelihood of sampling similar individuals from both populations is comparable to that of sampling them from within either population. The temporal samples were pooled for the determination of mean nucleotide sequence divergences between North Carolina and the other populations.

Heterogeneity analysis was performed on the pooled haplotype distributions from all of the bay scallop samples. One thousand Monte Carlo simulations yielded no  $\chi^2$  values exceeding the observed, indicating that the populations did not share a common gene pool. The two North Carolina populations were tested separately for heterogeneity using this method, with 987 simulations producing  $\chi^2$  values equal to or greater than the observed. This is consistent with the null hypothesis of a shared common gene pool, and as expected from the calculated divergence value, these two populations showed little temporal partitioning of variation. Tests for heterogeneity were likewise performed on geographically separate pairs of populations that shared a haplotype. In all of these, no  $\chi^2$  values higher than the observed were produced, indicating that significant heterogeneity exists between the populations.

Phenetic analysis by the unweighted pair-group method (UPGMA) performed on the mean nucleotide sequence divergences among samples of *A. irradians* illustrates the lack of dif-

TABLE 3.

*Argopecten irradians*. Summary statistics.

	MA	NC	NCT	NC <sub>pool</sub>	FL	RK
# individuals	26	27	21	48	27	34
# haplotypes	9	13	9	21	6	17
Haplotype diversity ( <i>h</i> )	0.87	0.74	0.63	0.69	0.74	0.91
% mean nucleotide sequence diversity ( <i>p</i> )	0.48	0.28	0.22	0.25	0.53	0.50

MA = New England; NC = Harker's Island, NC; NCT = NC + 1 yr.; NC<sub>pool</sub> = NC + NCT; FL = Crystal River, FL; RK = Rabbit Key, FL.

TABLE 4.

*Argopecten irradians*. Matrix of nucleotide sequence of divergences among populations, in percent.

	MA	NC	NCT	NC <sub>pool</sub>	FL	RK
MA	—	0.51	0.46	0.49	0.68	0.61
NC	0.13	—	0.25	—	0.53	0.58
NCT	0.11	<0.01	—	—	0.51	0.54
NC <sub>pool</sub>	0.12	—	—	—	0.52	0.56
FL	0.18	0.13	0.14	0.14	—	0.62
RK	0.12	0.19	0.18	0.19	0.11	—

Uncorrected above diagonal; below diagonal corrected for within-sample variation. MA = New England; NC = Harker's Island, NC; NCT = NC + 1 yr.; NC<sub>pool</sub> = NC + NCT; FL = Crystal River, FL; RK = Rabbit Key, FL.

ferentiation between the two North Carolina samples (Fig. 1). These appear to be approximately equidistant from the cluster formed by the Rabbit Key (RK) and Crystal River (FL) samples, and the New England (MA) population.

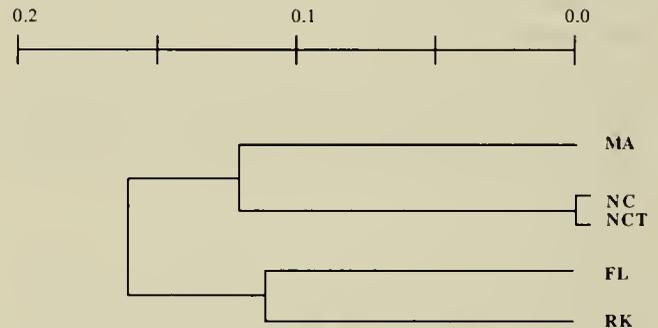


Figure 1. Dendrogram from UPGMA cluster analysis of five samples of *A. irradians*. Scale is percent nucleotide divergence. MA = New England; NC = Harker's Island, NC; NCT = NC + 1 year; FL = Crystal River, FL; RK = Rabbit Key, FL.

#### *A. gibbus*

In all, 51 calico scallops were screened with 6 restriction endonucleases, to produce 19 distinct mtDNA haplotypes (Table 5). Enzymes produced between 2 and 7 distinct fragment patterns, and none was invariant. A total of 60 fragments was visualized, and restriction site gains and losses were inferred from additive changes in fragment patterns. The different fragment patterns were accounted for with 3 to 12 sites, and 42 sites were scored in total. The size of the whole mtDNA molecule was estimated at 16.5 kb from the *AvaI* digests, and no indications of size polymorphism or heteroplasmy were apparent.

Five of the 19 haplotypes were shared between the two populations of calico scallops. The most common haplotype, aaaaaa, was present in both samples, in a total of 17 individuals. It comprised 36 restriction sites, accounting for an estimated 1.2% of the genome. The haplotype diversities of the Cape Canaveral and Apalachicola populations were both large at 0.79 and 0.92, respectively. Mean nucleotide sequence diversities were 0.54% for the Cape Canaveral sample, and 0.69% for the Apalachicola individuals (Table 6). Corrected and uncorrected nucleotide sequence divergences between the populations were 0.007% and

TABLE 5.

*Argopecten gibbus*. Composite haplotypes from two populations.

Haplotype	CA	AP
aaaaaa	12	5
aaaaac	1	
aadaaa	1	
aaeaaa	3	
adaaaa	1	3
babaaa	4	4
babaaf	1	
bacaaa	1	1
bafaaa	1	1
bbbbaa	1	
bcbabb	1	
aaaaad		1
aaaaag		1
aaaace		1
adeaaa		2
adeaac		1
aegaaa		1
babaab		1
bdbaaa		2
Total n	27	24

CA = Cape Canaveral, FL; AP = Apalachicola, FL. Restriction enzymes used: *Ava*I, *Ban*I, *Bgl*II, *Bst*EII, *Eco*RI, and *Hae*II.

0.621% respectively, indicating that most of the sequence divergence could be attributed to variation within the samples.

A Monte Carlo test for heterogeneity performed for the two calico scallop samples yielded 143 out of 1000 replicates with  $\chi^2$  values exceeding that calculated from the original data, suggesting that the populations are not significantly heterogeneous. That is, the null hypothesis that the two populations share a common gene pool could not be rejected.

The Cape Canaveral sample of calico scallops was screened with the full battery of eight restriction enzymes used in the bay scallop analysis. No similar banding patterns or shared fragments were observed between the two species.

DISCUSSION

*A. irradians*

Significant genetic heterogeneity was revealed among the four geographically isolated bay scallop populations. This result is consistent with morphological differentiation among the populations (Clarke 1965). No clear trends were apparent in the magnitudes of corrected nucleotide sequence divergences and geographic distribution. The New England sample was equally divergent from the pooled North Carolina sample (0.12%) and the Rabbit Key sample (0.12%), but was slightly more divergent from the Florida Gulf population (0.18%). The pooled North Carolina sample, however, was more divergent from the Rabbit Key scallops (0.19%) than from the Florida Gulf sample (0.14%). This raises the question of the validity of assigning subspecies designations on the basis of these data. Since within-sample diversities were so high, it is difficult to determine trends among populations that might be indicative of relatedness at the subspecies level.

Pairwise testing of geographically separate populations with the same taxonomic designation did not reveal any that shared a

common gene pool. Again this was likely due to the isolated nature of bay scallop populations, and the geographic distance separating the populations included in this study. The potential for sampling effects on these results should be considered, as error may have been introduced when scallops were collected. Removal of scallops from the field was performed by other researchers or culturists in all cases, and little is known about collection procedures used. It is assumed that the populations were effectively sampled with the removal of 30 to 50 individuals, but there remains the possibility that maternally-based patchy distributions were encountered within populations. Diversity values within populations were sufficiently high (0.63 to 0.91) that bias introduced by non-random sampling might be considered negligible. Inter-population comparisons of diversity would not be valid, however, particularly because two of the "natural" samples were actually derived from cultured populations. The New England sample, as described earlier, had been subjected to broodstock introductions from a number of Massachusetts sources. The degree to which this population had been inbred is not known. The Crystal River sample was only one generation removed from the source population, but the measured diversity within this sample cannot be said to approximate that of Crystal River bay scallops. In the cases in which the samples were derived from populations which had been subjected to hatchery breeding, one might expect to see a significant decrease in genetic diversity due to inbreeding and the potential for a genetic bottleneck. Notably, neither the New England nor the Crystal River samples showed diversities below those of samples collected from naturally settled populations.

Nucleotide sequence divergence data based on an analysis of mtDNA were not inconsistent with the findings of a study of allozyme variation in bay scallops (Marelli et al., in press). Marelli et al. (in press) found individuals of a North Carolina population of *A. i. concentricus* to be genetically intermediate to New England *A. i. irradians* and Florida *A. i. concentricus*. Genetic-distance values (Nei's D) among the subspecies of *A. irradians* were found to range between 0.047 and 0.188, with increased divergences between populations separated by greater geographic distance. The genetic distance between bay scallops from western Florida and North Carolina was no smaller than that between the North Carolina population and a sample of *A. i. irradians* from Massachusetts (Marelli et al., in press).

UPGMA analysis based on mtDNA nucleotide sequence divergences placed North Carolina and New England bay scallops in a cluster separate from the Florida Gulf and Rabbit Key samples (Fig. 1). This agrees with an UPGMA dendrogram based on al-

TABLE 6.

*Argopecten gibbus*. Summary statistics.

	CA	AP
# individuals	27	24
# haplotypes	11	13
haplotype diversity ( <i>h</i> )	0.79	0.92
% mean nucleotide sequence diversity ( <i>p</i> )	0.54	0.69
Nucleotide sequence divergence between CA and AP samples of <i>A. gibbus</i> , in percent:		
Uncorrected: 0.621		
Corrected for within-sample variation: 0.007		

CA = Cape Canaveral, FL; AP = Apalachicola, FL.

lozymes (Marelli et al., in press), which also placed a North Carolina bay scallop population in a cluster with samples from Massachusetts, Connecticut and Long Island, while samples from the Florida Gulf and Rabbit Key clustered separately. Based on these dendrograms, neither the mtDNA nucleotide sequence divergence data nor the allozyme data support the preferred classification of North Carolina bay scallops as *A. i. concentricus*, as traditionally determined by morphological characteristics. Standard errors on the branches of these dendrograms make them essentially indistinguishable, however, and qualitatively both show North Carolina samples to be intermediate to two major clusters grouped closely along the lines of their morphologically-determined taxonomic designations. It is informative then, to consider bay scallops from New England and Florida's west coast to be separate subspecies, inasmuch as they are morphologically and genetically distinct, but it is not clear which of these classifications is most aptly assigned to the bay scallops of North Carolina. Based on morphology, North Carolina bay scallops should continue to be classified as *A. i. concentricus*, whereas genetically there is no evidence to suggest that this is a more appropriate designation than *A. i. irradians*.

Clarke (1965) delineated the range of *A. i. concentricus* to include the region between New Jersey and North Carolina. Unfortunately, it has not been possible to apply RFLP or allozyme analysis to bay scallop populations from this region, as none remain to be sampled. It seems probable that the clinal variation that places North Carolina bay scallops intermediate to those of New England and the Gulf, would also be apparent in a New Jersey sample, with the latter possessing morphological and genetic characters intermediate to those of New England and North Carolina.

The distributions of haplotypes among scallops sampled from the same North Carolina population in two consecutive years (i.e., two generations) were very similar, consistent with the hypothesis that the temporally isolated populations shared a common gene pool. Of the haplotypes observed in the NC and NCT samples, only one (AACAAAA) was common to both, but this was found in 48 and 62% of the individuals, respectively. This finding permits more confidence in the interpretation of results from geographically-separated populations. Since temporal variation was found to be relatively small, the high levels of variation within the geographically isolated samples may be taken as characteristic of their respective populations—that is, they are not just "noise" in the signal that might prevent characterization and distinction of populations with these genetic data. This finding also holds implications for the question of bay scallop larval dispersal ability. It is apparent from the lack of temporal variation in these samples that the scallops recruited to this population in the two years examined originated from the same or similar broodstock. One manner in which this temporal homogeneity may be maintained is by retention of larvae from a local spawning event, so that those recruited to the population are offspring of the previous generation. For a species that is short-lived and essentially annual, this may be used as a measure of recruitment success.

Heterogeneity analysis indicated that *A. i. taylorae* from Rabbit Key, Florida and the Crystal River population of *A. i. concentricus* did not share a common gene pool, although the Rabbit Key scallops clustered with the Crystal River sample in the UPGMA analysis. The nucleotide sequence divergence of 0.12% between the two populations would not seem to justify a separate subspecies status for the Rabbit Key scallops. These are at least as related to

the Crystal River sample as is the sample designated *A. i. concentricus* from North Carolina. As such, the suggestion that *A. i. taylorae* was described from juvenile *A. i. concentricus*, is supported (Marelli et al., in press). Morphological variation between the populations may be limited to phenotypically plastic characters that are expressed differently in the two regions.

#### *A. gibbus*

Heterogeneity analysis of the calico scallop samples was consistent with the hypothesis of a shared, common gene pool for the two populations. This supports the suggestion that *A. gibbus* larvae are capable of wide dispersal (Kirby-Smith 1970, Krause et al. 1994). Entrainment in the Loop Current of the eastern Gulf of Mexico and/or the Gulf Stream might provide transport such that larvae from individuals spawned in the northern Gulf of Mexico could feasibly recruit to populations on the Atlantic coast of Florida. Transport in the opposite direction, from the Atlantic into the Gulf, does not seem as likely, but it is apparent that sufficient gene flow occurs to prevent divergence of these geographically separate populations by the mechanisms of natural selection and genetic drift.

Genetic divergences among populations of bay and calico scallops revealed few clear trends or biogeographic patterns, as have been observed for the oyster and horseshoe crab (Saunders et al. 1986, Reeb and Avise 1990). An UPGMA cluster analysis for the haplotypes observed in the calico scallop did not reveal two distinct phenetic groups characterizing the Atlantic and Gulf coast populations, as it did for *Crassostrea virginica* (Reeb and Avise 1990). If a break in the distribution of mtDNA haplotypes exists for the calico scallop, it may only be detectable at sites north of Cape Canaveral.

Clear differences in the mitochondrial DNA of bay and calico scallops were detected in the course of this study, indicating that RFLP analysis could provide a fairly simple diagnostic technique for distinguishing the two species. Both are marketed primarily as a shucked product—adductor muscle only—and in this form are virtually identical in appearance. RFLP analysis with a small battery of restriction enzymes should provide a useful tool for distinguishing the meats of the two species, as might be desirable for the regulation of the separate fisheries.

Perhaps the most interesting feature revealed in the comparative genetic analysis of the two species is the different patterns of geographic variation which correspond to differences in life history and recruitment dynamics. The calico scallop, which dwells offshore, and whose distribution is considered fairly continuous around the Florida peninsula, showed little genetic differentiation between two populations separated by considerable geographic distance. The bay scallop, conversely, showed heterogeneity among all of the populations examined. This reflects the relative isolation of bay scallop populations, which by virtue of limited habitat and a different recruitment strategy, maintain distinct haplotype distributions.

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## SELECTION RESPONSE AND HERITABILITY ESTIMATES FOR GROWTH IN THE CHILEAN OYSTER *OSTREA CHILENSIS* (PHILIPPI, 1845)

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**ABSTRACT** Oyster parent stock was taken from the 1987 natural spatfall of *Ostrea chilensis* collected on plastic plates in the Quempillén River Estuary of southern Chile. From the 40-month-old cohort 5,030 oysters were monitored for live weight and shell length. Divergent selection was carried out by applying a selection intensity of  $\pm 1.755$  for the trait "live weight". Five subgroups each of high selected oysters, low selected oysters and an unselected control group were conditioned in 15 150-liter tanks. A highly significant correlation ( $r = 0.94$ ,  $N = 5,030$ ) was found between the traits "live weight" and "shell length". Juveniles from the 10 selected and 5 control subgroups were individually tagged. Live weight and shell length were monitored after 8, 14 and 27 months in 200 oysters from each subgroup. The phenotypic serial correlations for both traits indicate that either 8 or 14 months of age can be appropriate for the selection of parents to be used in an *O. chilensis* breeding program. The ANOVA results show a significant difference, in both traits, between the high and low selected groups at 8, 14 and 27 months of age. The heritability estimates for increasing the trait "live weight" ranged between  $0.43 \pm 0.18$  and  $0.69 \pm 0.11$  while the heritability estimates for decreasing the same trait ranged between  $0.24 \pm 0.06$  and  $0.35 \pm 0.08$ . Mass selection appears to be a promising technique for the improvement of the Chilean oyster broodstocks.

**KEY WORDS:** Selection, heritability, *Ostrea chilensis*, growth

### INTRODUCTION

The Chilean oyster, *Ostrea chilensis* Philippi 1845, represents an economically important resource in southern Chile. Although its culture has been very important during the last two decades, particularly since demand by the North-American market has increased (Lepez 1983, Chaparro 1990, Toro and Chaparro 1990, Bustos et al. 1991), it has been restricted by the lack of a reliable source of natural seed. Also, the very slow growth rate of this species in southern Chile, due to the very short growing season (from November to February), make it nearly unprofitable for oyster farmers. These oysters are marketable at a shell length of about 50 mm, which is attained after four to five years of growth in natural beds or 30 months in culture (Winter et al. 1984, Kino and Valencia 1990). Very little has been published on the genetic improvement of the Chilean oyster. Control of the complete life cycle, which is required for any type of direct genetic improvement (Hershberger et al. 1984), has been accomplished only recently (DiSalvo et al. 1983). There are several reports in the literature addressing the importance and potential of genetics in aquaculture (Moav 1976, Newkirk 1983, Gjedrem 1983, Gjerde 1986, Guíñez 1988, Toro and Newkirk 1990a, Beaumont and Fairbrother 1991, Bustos et al. 1991). Increasing production efficiency, reducing generation time and/or improving survival rate, will reduce the negative effects of short growing seasons in high latitudes (Newkirk 1980).

Growth rate is considered the most economically important trait to be improved in a selection program. Traits of economic importance are usually determined by a large number of genes each having a small contribution and under considerable environmental influence (Newkirk 1980, Lande 1982). Thus, these kind of traits are best studied through the quantitative genetics theory, which reduces the most important features of complex genetic systems to a relatively few variables that can be estimated from phenotypic measurements (Falconer 1981, Lande 1982).

A very high intrapopulation variation of the growth rate of *O. chilensis* has been reported in the literature (DiSalvo and Martinez 1985, Toro and Varela 1988, Bustos et al. 1991). However, there have been only a few reports on genetic parameters (Guíñez and Galleguillos 1985, Guíñez et al. 1986) or artificial selection for growth rate (Bustos et al. 1991, Toro 1992) in *O. chilensis*. The fact that there is phenotypic variation in growth rate suggests that there might be significant genetic differences which could be exploited in a selection program to produce faster growing oysters. A selection program requires the estimation of an important genetic parameter such as heritability ( $h^2$ ) for economically important traits. A review of the literature shows that heritability estimates in oysters have been mainly restricted to larval and juvenile stages. For *Crassostrea gigas*, Lannan (1972) reports  $h^2$  estimates of 0.31 for larval survival, and a range between 0.31 to 1.17 for various spat traits at 12 months. However, these  $h^2$  values were estimated using a fullsib analysis and might be biased due to maternal or non-additive genetic effects. Longwell and Stiles (1973) report an  $h^2$  of 0.24 for larval growth rate at 14 days. This estimation was made using a halfsib analysis with *Crassostrea virginica* families. Haley et al. (1975) and Newkirk et al. (1977) also describe heritabilities for larval growth rate in *C. virginica* by analyzing full and halfsib families. Their estimations range between 0.25 and 0.50. Losee (1978) reports  $h^2$  estimates, based on halfsib families in *C. virginica*, for larval size of between 0.40 and 0.55 and for juveniles six weeks after setting of between 0.3 and 0.7. In adults, Hadley et al. (1991) report heritability estimates for growth rate in *Mercenaria mercenaria* at 2 years of age that range between  $0.42 \pm 0.10$  and  $0.43 \pm 0.06$ . Toro and Newkirk (1990a) report heritability values for growth rate in *Ostrea edulis* that range between  $0.112 \pm 0.04$  and  $0.243 \pm 0.12$ . A high heritability value for a particular trait indicates that a large proportion of the trait is associated with additive gene action, and that genetic progress can therefore be achieved through mass selection (Newkirk et al. 1977). Mass selection has been used to improve production traits

in several aquatic species (Gjedrem 1983). This method of selection entails selection of individuals based on their phenotypic merits and has given different results with different species.

One major difficulty which occurs at the beginning of a selection program is the accurate identification of genetically superior oysters at the earliest possible stage. When planning a selection program, phenotypic correlations between growth rate at earlier stages of the life cycle and at near the market size is of great help in identifying the best individuals for selected parents as early as possible in their life cycle.

Knowledge of these correlations will allow a reduction in the generation interval and thus, a higher gain per year (Toro and Newkirk 1990b). However, reports for other oyster species indicate that there is a very low correlation between growth rate during earlier months of life and growth rate at later stages (Wilkins 1981, Newkirk and Haley 1982b).

The present study represents one portion of a large-scale breeding program to improve the growth rate of the Chilean native oyster *O. chilensis*. This study was designed to evaluate the divergent selection (Falconer 1981) of *O. chilensis* that arises as a response to a differential selection applied to the parental population. Divergent selection (heredity lines to increase and decrease the trait values) is of great utility to reduce the standard error of the heritability (Falconer 1963, 1981). Also, a control line permits the presence of asymmetry of response between the two lines to be established.

## MATERIALS AND METHODS

The parent stock was taken from the 1987 spatfall of the wild population of *O. chilensis* in the Quempillén River Estuary, located on Chiloé Island, southern Chile (41°51'S; 73°46'W). The spat collected on plastic plates were grown on long lines in the estuary until 8 months of age (September 1988). At that time, 8,000 juveniles were randomly taken, labelled by gluing numbers onto the shells (Toro and Newkirk 1990b) and allowed to grow in suspended trays (150 oysters/m<sup>2</sup>) (Toro and Varela 1988). At 40 months of age (June 1991), a frequency distribution for the traits "live weight" and "shell length" from a random sample of 5,030 oysters was carried out. Both traits presented a normal distribution ( $p > 0.05$ , Kolmorov Smirnov, Sokal and Rohlf 1981, Wilkinson 1991).

Divergent selection was applied in order to obtain low selected and high selected lines for the trait "live weight". The selection intensity applied was +1.755 and -1.755, corresponding to the largest and smallest 10% of the trait distribution. A similar number ( $n = 500$ ) of average-sized animals were segregated as control-line parents. Each group of 500 individuals corresponding to the selected and control parents were randomly separated into five subgroups of 100 each and conditioned for spawning on July 4. The basic statistics for the population ( $N = 5,030$ ) and the selected and control lines are given in Table 1.

During conditioning, the water temperature was raised from the ambient temperature (11°C) to 18°C in 4 weeks. The salinity and pH of the water during conditioning ranged between 29 and 30 ppt and 7.2 and 8.4, respectively. The filtered- and UV-sterilized sea water was changed daily and a mixture of *Isochrysis galbana*, Clone T-iso and *Chaetoceros gracilis* Shüt (500,000 cel/mL) was added continuously. The ration of microalgae was calculated ac-

TABLE 1.

Basic statistics for the traits "live weight" (g) and "shell length" (mm) for the 40-month-old cohort (5,030) of *O. chilensis*, used as the parental population.

N	Population 5030	High 500	Low 500	Control 500
<b>LIVE WEIGHT</b>				
Mean	25.75	38.10	12.26	26.18
S.D.	8.2	5.2	1.9	5.4
C.V. (%)	31.8	13.6	15.5	20.6
<b>SHELL LENGTH</b>				
Mean	43.66	56.97	32.35	45.18
S.D.	7.4	4.5	3.1	4.1
C.V. (%)	16.9	7.9	9.5	9.1

Mean, standard deviation (S.D.) and coefficient of variation (C.V.) for the overall parental population, and high, low and unselected control groups, respectively.

ording to Winter et al. (1984) and Chaparro (1990). In the present study, the authors used 1.5% of the oyster dry weight in microalgae dry weight, using the relationship between dry weight (g) and shell length (mm) ( $r = 0.91$ ,  $p = 0.00$ ) following Strickland and Parsons (1972).

At the time of the water change all groups were checked for released larvae (using a 250  $\mu$ m mesh). The conditioning period was 8 weeks, two weeks more than that reported in the literature for *O. chilensis* (Wilson 1988). The longer period in this study was probably necessary due to an accidental decrease (40%) in the microalgae ration during a few days. However, this had no effect on the size of the released larvae (average length = 480  $\mu$ m; SD = 21.6,  $N = 400$ ), which was similar to that reported by Gleisner (1981), and by Winter et al. (1983). After 5 weeks of conditioning, 5 sets of 8 plastic plates of 150  $\times$  150 mm were submerged in the tank. Larvae were released by the female oysters after 8 weeks (September 3 to 6) and settlement occurred within the first 24 hours, confirming the very short period of free-swimming larvae in this species. After labelling, all the plastic plates were transferred to a common rearing tank of 1,500 l at  $16 \pm 1^\circ$ C. The water change was made every other day and the microalgae ration was increased (50 l per day with a concentration of  $1.8 \times 10^6$  cel/mL). After 15 weeks of growth in the laboratory, the juveniles were individually labelled and transferred randomly to pearl nets deployed at three different depths in the estuary. "Live weight" and "shell length" were monitored at 8, 14 and 27 months using an A&D balance (0.01 g) and a vernier caliper (0.1 mm). Analyses of Variance (ANOVA) including the effects of depth, group (high and low selected) and their interaction were carried out for "live weight" and "shell length." Heritability estimates and their standard errors were calculated according to Becker (1984) and Hadley et al. (1991).

## RESULTS AND DISCUSSION

A high phenotypic correlation between "live weight" and "shell length" was found ( $r = 0.94$ ,  $N = 5030$ ,  $p = 0.000$ ). This result can be very important when considering the possible use of indirect selection, utilizing "shell length" as the trait to be selected instead of "live weight" because it is easier to measure.

However, an estimation of the genetic correlation between both traits is needed in order to estimate the relative efficiency of indirect, compared to direct, selection (Turner 1969).

An average of about 3,000 spat settled on the plastic plates placed in each of the 15 tanks. A greater percentage of the eyed-larvae, unfortunately, settled on the tank walls. This could have been avoided by using vaseline on the internal surfaces of the tanks, however, in the present study this loss of spat do not represent a real problem, because the settlement was random and a denser settlement on the plastic plates may have produced competition for space among the spat.

Growth data at 8, 14 and 27 months of age for the traits "live weight" and "shell length" are presented in Table 2. The means of the groups (Control, High and Low) are different, however, the coefficients of variance are quite similar among the groups with values ranging from 10 to 26 for "live weight" and from 16 to 32 for "shell length" (Table 2).

The ANOVA results (Table 3) show that for "live weight" and "shell length", there is a significant effect of the group source of variance (High and Low), with no significant effect of depth at 8, 14 and 27 months of age. Toro and Newkirk (1991) reported that in the Quempillen River Estuary there was a significant effect of depth on the growth rate (shell height) of *O. chilensis*, however, the specific location in the estuary and the culture system used in the present study differ from those used by the above mentioned authors. Also, a significant effect of the subgroup within group source of variation was detected in the oysters used in this study at the 7th and 15th week of age (Toro et al. 1992), however the effect showed no correlation with the parental values for the trait "shell length". This result can be explained by the use of a random distribution of the individuals among pearl nets, after 15 months of age.

A significant response to selection for the direct selected trait "live weight" was found after 8, 14 and 27 months of age (Table 4). The difference in growth between the two lines in *O. chilensis* indicates that there is a significant genetic component which can be used in a selection program. The standardized responses fluctuated between +1.40 and -0.94 units of S.D. from the control. A correlated response for the trait "shell length" was detected (Table 5). These results are in accordance with those reported by Bustos et al. (1991) where a positive response to selection was found to increase the trait "shell length" in *O. chilensis*. Toro and Newkirk (1991) also found a significant response to reduce the same trait, using a Low selected line with an unselected control line in *O. chilensis*.

The values of response to selection of the traits expressed in percentage, 13 to 33% (Tables 4, 5), are in accordance with those

given by Newkirk and Haley (1982a) in *O. edulis*. These authors reported a large response to selection for growth to market size with an average of 23% of gain over the controls.

In the present study, it is possible to observe an asymmetry of response for the divergent lines selected. According to the standardized index (Tables 4, 5) there is a greater response to increasing the traits. The genetic basis of this result is very difficult to establish at this stage, but according to Falconer (1981), there are many sources of variation that can produce this asymmetry in response. Bondari (1983, 1986) also reports that divergent selection yields a better response for increasing the body weight in the Channel catfish (*Ictalurus punctatus*).

The correlated response found for the trait "shell length" suggests a positive genetic correlation between the two traits. Very high genetic correlations (close to unity) between "live weight" and "shell length" have been reported ( $r_a = 0.995$ ) for *O. edulis* (Toro and Newkirk, 1990a).

Mass spawning was used in the present study in order to ensure success in obtaining offspring from the selected groups. Some constraints of the limited hatchery facilities also indicated that mass spawning be used. Although, when using mass spawning, one can not know how many oysters contribute genes to the offspring obtained, some estimation of the female contribution can be made by keeping track of the number of eyed-larvae released in each tank. Fecundity in *O. chilensis* ranges between 10,000 and 115,000 larvae per season per adult with an average of 60,000 (Gleisner 1981, Lepez, 1983, Winter et al. 1983, Kino and Valencia 1990). Thus, by the average number of eyed-larvae released in each tank,  $1.95 \times 10^6$ , it can be assumed that at least 30 females contributed larvae to each tank. This may be an underestimation because, in this species, some of the eyed-larvae released from the female oyster will be set in as little as 5 minutes (DiSalvo et al. 1983). In order to avoid this problem, the "pair mating design" discussed by Newkirk (1986), is now being used to produce fullsib families of *O. chilensis*.

The realized heritability estimates ( $h^2$ ) for increasing and reducing the traits "live weight" and "shell length" ranged, at 27 months of age, between 0.43 and 0.70 and between 0.24 and 0.36, respectively (Table 6). These heritability values are lower than those reported by Newkirk and Haley (1982a) in *O. edulis*, Lannan (1972) in *C. gigas* and Stromgren and Nielsen (1989) in *Mytilus edulis*, however, they are higher than those reported by Toro and Newkirk (1990a) in *O. edulis* and Toro and Newkirk (1991) in *O. chilensis*. The heritability values estimated in the present study are in accordance with those reported by Wada (1986) in *Pinctada fucata martensii* ( $h^2 = 0.47$  for shell width and 0.35 for shell convexity), Hadley et al. (1991) in *M. mercenaria* ( $h^2 = 0.43$  for growth rate) and also in line with values for production traits in livestock (Van Vleck 1987).

An estimation of heritability for economically important traits such as "live weight" and "shell length" is essential, in a long term breeding program, to enhance the growth rate of oysters. Heritability values are necessary for the purpose of predicting the genetic gain by selection. According to Falconer (1981), and by experimental data obtained in the Japanese pearl oyster *P. fucata martensii* (Wada 1986) heritability normally does not decline during the first five to ten generations of selection when the initial, effective population size is large. According to this assumption (Falconer 1981) in a second generation of selection in *O. chilensis*, using a selection intensity of 1.755 (upper 10% of the high selected line), the 27 month old heritability estimates and similar

TABLE 2.

Basic statistics for the traits "live weight" (g) and "shell length" (mm)  $\pm$  standard deviation (S.D.) at 8, 14 and 27 months of age for the High, Low and Control groups.

Trait	Age	High	Control	Low
Live weight	8	0.32 $\pm$ 0.04	0.24 $\pm$ 0.06	0.20 $\pm$ 0.02
	14	1.61 $\pm$ 0.26	1.27 $\pm$ 0.34	1.12 $\pm$ 0.29
	27	27.64 $\pm$ 3.06	22.41 $\pm$ 3.72	18.89 $\pm$ 3.27
Shell length	8	14.80 $\pm$ 3.12	11.63 $\pm$ 3.76	10.41 $\pm$ 3.07
	14	25.63 $\pm$ 4.08	20.59 $\pm$ 4.21	17.68 $\pm$ 3.91
	27	46.83 $\pm$ 6.42	41.41 $\pm$ 5.66	37.31 $\pm$ 5.52

TABLE 3.

ANOVA Table. Mean Squares (MS) for "live weight" and "shell length" after 8, 14 and 27 months of age for the effects of Depth (0.5, 1.0 and 1.5 m), Group (high and low) and their interaction (Depth \* Group).

Effect	d.f.	8		14		27	
		MS		MS		MS	
		Weight	Length	Weight	Length	Weight	Length
Depth	2	3.271	0.825	24.264	4.013	98.825	9.692
Group	1	54.632*	5.212*	235.911**	27.740**	312.683**	97.409**
Depth * Group	2	3.482	0.956	77.049	13.338	95.233	15.952
Residual	2570	0.372	0.085	1.365	0.189	1.843	0.518

\*  $P < 0.05$ ; \*\* $P < 0.001$ .

environmental conditions, an additional gain of 6.18 g and 5.84 mm would be expected, thus, shortening the time to market size.

Another important point within a selection program is the age at which the superior individuals should be selected as parents for the next generation. The phenotypic correlations between the values of the traits at near market size and the values at early stages are shown in Table 7. Using the coefficient of determination ( $r^2$ ), we can conclude from these results that the "live weight" at 27 months was determined with 77% and 83% accuracy by the "live weight" at 8 and 14 months, respectively, and that the "shell length" at 27 months of age was determined with 93% and 75% accuracy by the "shell length" at 14 and 8 months of age, respectively. These results indicate that either 8 or 14 months can be an appropriate age to select parents to be used in a breeding program

with *O. chilensis*. At that time, the oyster size is indicative of its performance to market size. By reducing the generation time it may be possible to make more progress on a yearly basis even though the progress on a per generation basis is less than the maximum possible. We should balance the length of generation time against the precision in determining the true value of individuals (Lerner and Donald 1966, Longwell 1976, Haley and Newkirk 1978). It is more efficient to select the broodstock at market size or at least near enough to market size to have the highest probability of choosing the best individuals. However, the high costs of maintaining the broodstock for 3 to 4 years instead of 1 or 2 is another point to consider. The broodstock must be grown under controlled conditions to ensure that environmental effects are random among the stock.

TABLE 4.

*Ostrea chilensis*. Selection intensity and standardized response to selection for the trait "live weight" (g) at 8, 14 and 27 months of age.

	Group Means			Population	
	High	Low	Control	Mean	S.D.
PARENTS:					
selected	38.10	12.26		25.75	8.20
control			26.18	25.75	8.20
Standardized Index	1.51	1.65	0.05		
Adjusted Index	1.45	1.70	0.00		
Response to					
Divergent Selection					
8 Months					
Selected groups	0.32	0.20		0.24	0.06
Control group			0.24	0.24	0.06
Standardized Index	1.33	-0.67	0.00		
Gain (%)	33.33	-16.66			
14 months					
Selected groups	1.61	1.12		1.27	0.34
Control Group			1.27	1.27	0.34
Standardized Index	1.00	-0.44	0.00		
Gain (%)	26.77	-11.81			
27 months					
Selected groups	27.64	18.89		22.41	3.72
Control Group			22.41	22.41	3.72
Standardized Index	1.40	-0.94	0.00		
Gain (%)	23.33	-15.71			

TABLE 5.

*Ostrea chilensis*. Selection intensity and standardized response to selection for the trait "shell length" (mm at 8, 14 and 27 months of age).

	Group Means			Population	
	High	Low	Control	Mean	S.D.
PARENTS:					
selected	56.97	32.35		43.66	7.40
control			45.18	43.66	7.40
Standardized Index	1.80	1.53	0.21		
Adjusted Index	1.59	1.73	0.00		
Response to					
Divergent Selection					
8 Months					
Selected groups	14.80	10.41		11.63	3.76
Control group			11.63	11.63	3.76
Standardized Index	0.84	-0.32	0.00		
Gain (%)	27.25	-10.49			
14 months					
Selected groups	25.63	17.68		20.59	4.21
Control Group			20.59	20.59	4.21
Standardized Index	1.19	-0.69	0.00		
Gain (%)	24.47	-14.13			
27 months					
Selected groups	46.83	37.31		41.41	5.66
Control Group			41.41	41.41	5.66
Standardized Index	0.95	-0.72	0.00		
Gain (%)	13.08	-9.90			

TABLE 6.

*Ostrea chilensis*. Realized heritability estimates and their standard error (S.E.) for increasing and reducing the traits "live weight" and "shell length" at 8, 14 and 27 months of age.

Live Weight	Age	h <sup>2</sup>		S.E.	h <sup>2</sup>		S.E.
		High	±		Low	±	
	8	0.69	±	0.11	0.35	±	0.08
	14	0.55	±	0.10	0.24	±	0.06
	27	0.43	±	0.18	0.29	±	0.13

Shell Length	Age	h <sup>2</sup>		S.E.	h <sup>2</sup>		S.E.
		High	±		Low	±	
	8	0.70	±	0.10	0.27	±	0.06
	14	0.63	±	0.09	0.36	±	0.07
	27	0.45	±	0.12	0.31	±	0.11

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TABLE 7.

Pearson correlation coefficients between the values of the traits "live weight" and "shell length" at 27 months of age and the values for the same traits at 14 and 8 months of age for the groups High selected (N = 734), Low selected (N = 585), Control (N = 1,260) and the total population (F1) of *O. chilensis* included in the selection program (N = 2,579).

Group	Live Weight		Shell Length	
	8	14	8	14
High	0.858**	0.866**	0.799**	0.936**
Control	0.852**	0.893**	0.828**	0.853**
Low	0.725**	0.838**	0.787**	0.883**
Population	0.886**	0.916**	0.875**	0.968**

\*\*  $P < 0.001$ .

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## A BIBLIOGRAPHY OF LITERATURE ON THE CHILEAN OYSTER *OSTREA CHILENSIS* (PHILIPPI, 1845)

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**ABSTRACT** A bibliography of 114 papers on the biology and cultivation of the Chilean oyster (*Ostrea chilensis* Philippi 1845) is presented to become a valuable tool to scientists and oysters farmers. It is the first bibliography dedicated to this species.

**KEY WORDS:** Chilean oyster, *Ostrea chilensis*, bibliography

In Chile, except for the experimental introduction of the Pacific oyster, the Chilean native oyster is the only oyster species of commercial importance, and it has been farmed since 1975 (Chanley and Chanley 1991). Several new generic names have been proposed for the Chilean native oyster in recent years. Initially, and in most of the references, this species is cited as *Ostrea chilensis* Philippi 1845; later was assigned to the genus *Tiostrea* as *Tiostrea chilensis* by Chanley and Dinamani (1980) and subsequently reassigned to the genus *Ostrea* as *Ostrea (Eostrea) puelchana* by Harry (1985) and most recently proposed as *O. puelchana* D'Orbigny 1846 by Carriker et al. (1994). Because the taxonomy of the oysters is still not very clear and Carriker et al. (1994) proposed *O. puelchana* as a synonym for *O. chilensis* despite the presence of some significant differences in the reproductive biology of these oysters (Fernandez Castro 1988, Pascual et al. 1992, Toro and Chaparro 1990), I will continue using *O. chilensis* Philippi, 1845 when referring to the Chilean native oyster.

The reproductive biology of the native Chilean oyster is characterized by the very long incubation period of the embryos which may last 50 days or longer, a large egg diameter (average = 250  $\mu\text{m}$ ), a reduced number of embryos/incubation period (average = 70,000) and a very short free larval stage (5 min—few hours) (DiSalvo et al. 1983, Winter et al. 1984). The extremely short period of pelagic larvae is one of the most important features of this species (Walne 1963). The females brood the larvae until they are

eyed and ready to metamorphose with an average size of 490  $\mu\text{m}$  (Walne 1974). Therefore, in controlled seed production of this species, it is not necessary to deal with the difficulties of feeding and handling the larvae (DiSalvo et al. 1984).

The growth rate of *O. chilensis* is relatively slow, especially at the low water temperatures in southern Chile (DiSalvo et al. 1983). Oysters reach market size after three years of growth in suspended trays, four years in suspended "cultch" system (Toro and Varela 1988) or after five years in bottom culture, with a shell length of about 50–60 mm (Solis 1967, Padilla et al. 1969). If this species can reach market size in a shorter period of time, it could become important on world markets. This species has already been exported to the markets of the following countries: Argentina, Brasil, Venezuela, Holanda, and España. Since 1988 in the United States, *O. chilensis* is highly acceptable (Avila et al. 1994). Presently, production could be increased considerably for domestic markets. The local demand for Chilean oysters exceeds the supply (Gutierrez 1984, Chanley and Chanley 1991).

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## FINE STRUCTURE OF THE DIGESTIVE TUBULE OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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**ABSTRACT** The fine structure of the digestive tubules of the eastern oyster, *Crassostrea virginica* (Gmelin 1791), has been studied by conventional transmission electron microscopy. Due to the paucity of information concerning the oyster digestive tubule, this research is necessary to achieve a fundamental understanding of its nutritional and physiological functions. Ultrastructural observations revealed that oyster digestive tubules are composed of three basic cell types: digestive cells, flagellated basophil cells, and non-flagellated basophil cells. These three cell types rest on a thin basement membrane containing irregularly spaced droplets of lipids and bundles of smooth muscle fibers. The most abundant cell type is the digestive cell. Digestive cells form a simple columnar epithelium between crypts, which contain the basophil cells. Digestive cells are characterized by well-developed microvilli, numerous microvesicles, and three distinct types of membrane-bound macrovesicles. These macrovesicles appear to represent the lysosomal-vacuolar system described in other bivalves. Non-flagellated basophil cells are characterized by a conspicuous nucleolus, an extensive granular endoplasmic reticulum, a well-developed Golgi apparatus, and several secretory-type vesicles in the sub-apical portion of the cell. These features are consistent with a cell specialized for the extracellular secretion of enzymes. By contrast, the flagellated basophil cells are characterized by the lack of well-developed organelles. The conspicuous feature of this cell type is the presence of a single, long flagellum. Although this cell type has been previously considered to be generative in nature, no evidence of mitotic activity was found.

**KEY WORDS:** *Crassostrea virginica*, digestive tubule, digestive cell, basophilic cell

### INTRODUCTION

The digestive diverticula of bivalves consist of numerous blind-ending tubules which connect with the stomach by way of main ducts and secondary ducts (Yonge 1926, Shaw and Battle 1957, Owen 1970). Light microscopy has revealed that the epithelium of the digestive tubule in bivalves is composed of two cell types (Yonge 1926, Mansour and Zaki 1946, Shaw and Battle 1957). Digestive cells, or secretory-absorptive cells, are believed to be responsible for the intracellular digestion of food material, and are characterized by a well-developed system of lysosomes. The dark-staining basophilic cells, or generative cells, are found within the crypts of the digestive tubule. Following the work of Yonge (1926), most investigators believed that the function of the smaller crypt cells is to replace the digestive cells when they are cast off (Shaw and Battle 1957, Galstoff 1964, Eble 1966, Sumner 1966, Owen 1970).

Ultrastructural studies on the digestive tubules of bivalves have altered many of these views on the structure and function of the basophilic crypt cells. For example, two different types of basophilic crypt cells have been identified in the freshwater bivalve, *Anodonta anatina* (Linnaeus 1758) (Sumner 1966); the marine cockle, *Cardium edule* (Linnaeus 1767) (Owen 1970); and the Indian estuarine bivalve, *Meretrix meretrix* (Linnaeus 1799) (Pal et al. 1990). Also, the basophilic crypt cells of many species closely resemble cells involved with the secretion (excretion) of extracellular enzymes (Sumner 1966, McQuiston 1969, Owen 1970, 1973, Pal 1971, Huca et al. 1982, Pal et al. 1990). Furthermore, the lack of observed mitotic figures in basophilic cells casts doubt on their role as a progenitor of digestive cells (Pal et al. 1990).

The purpose of this present work is to describe the ultrastructural details of the functional cell types found in the digestive

tubule of the eastern oyster, *Crassostrea virginica* (Gmelin 1791), and to compare the morphological features of the cell types found in oysters to those found in other species of bivalves. This work was undertaken because of the paucity of data concerning the fine structure of digestive tubules in oysters.

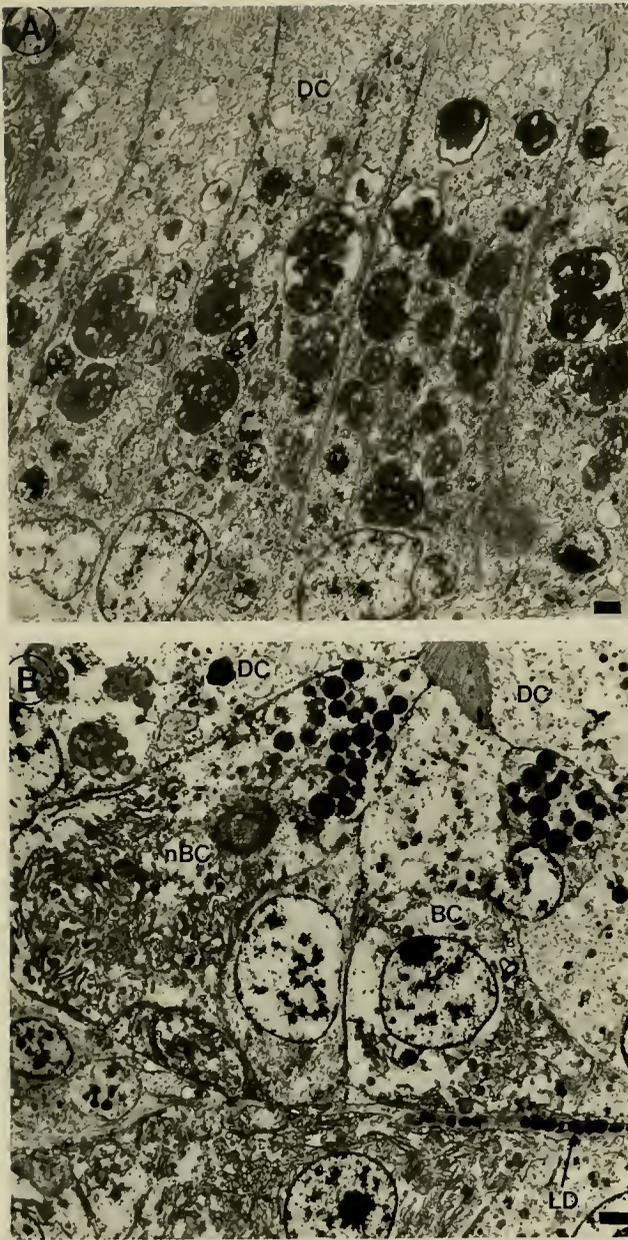
### MATERIALS AND METHODS

Oysters of uniform shell length (5.0–9.0 cm) were obtained during low tide on 10 October 1992, 15 January 1993, 1 April 1993, and 21 July 1993 from Clambank Creek, North Inlet, SC. Oysters were immediately prepared for histological examination. Cubes of fresh digestive gland tissue measuring 0.5–1.0 mm were cut under a fixative of 2.0% glutaraldehyde buffered in artificial seawater (Instant Ocean, Mentor, OH) at 4°C. Following 2 days of fixation, the tissue was washed in filtered artificial seawater at 4°C, post-fixed in 1.0% osmium tetroxide for 30 minutes, and rinsed in artificial seawater. Then, the tissues were dehydrated and embedded in epon. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963), and examined on a Hitachi 8000 transmission electron microscope operating at 100 kilovolts.

### RESULTS

No seasonal differences were observed in the structure of the digestive tubules of oysters collected from Clambank Creek; therefore, the following description is representative of all seasons sampled.

The digestive tubule in oysters is composed of three distinct cell types. The most abundant cell type is the digestive cell (Fig. 1A). Digestive cells form a simple columnar epithelium between the crypts, which contain the basophilic cells. Two types of ba-



**Figure 1.** Cells of the oyster digestive tubule. All bars = 1.0  $\mu\text{m}$ . (A) Cross-section through the oyster digestive tubule showing several columnar digestive cells (DC) (4,700 $\times$ ). (B) Cross-section through the crypt of an oyster digestive tubule showing the two types of basophil cells: non-flagellated basophil cells (nBC) and flagellated basophil cells (BC). On either side of the crypt, digestive cells are present. Note the presence of lipid droplets (LD) in the basement membrane surrounding these basophilic crypt cells (5,000 $\times$ ).

sophilic cells are present within these crypts: non-flagellated basophil cells and flagellated basophil cells (Fig. 1B). At their basal ends, these three cell types appear to rest on a thin basement membrane containing irregularly spaced droplets of lipids and bundles of smooth muscle fibers. Muscle fibers oriented in both the longitudinal and circular planes are present within each bundle.

The tall, columnar digestive cells possess an apical plasma membrane which bears long, well-developed microvilli (Fig. 2A). Within these microvilli, microfilaments are often evident. The apical plasma membrane exhibits active pinocytosis, as suggested

by the frequent small indentations or pits. These pits are bulb-shaped, and their inner membrane stains darkly. The lateral plasma membrane of adjacent digestive cells are sequentially linked through desmosomes, extensive septate desmosomes, and tight junctions. Basally, the lateral plasma membranes of adjacent cells occasionally appear separated from each other.

The most conspicuous feature of this cell type is the presence of the numerous membrane-bound microvesicles and macrovesicles, which comprise the lysosomal-vacuolar system. Microvesicles of variable electron opacity can be found throughout the cytoplasm; however, a disproportionate number occur in the sub-apical region of the cell (Fig. 2A). Many of these microvesicles have membranes which stain similarly to the inner membrane of the pits. These particular microvesicles probably represent pinocytotic vesicles.

There are primarily three types of macrovesicles found in the digestive cells of *C. virginica*. Type 1 macrovesicles are characterized by the "clear" appearance of their contents; however, minimal quantities of granular and membranous material may occasionally be present (Fig. 2B). These macrovesicles occur almost exclusively in the apical half of the cell, are irregular in shape, and their bordering membrane stains lightly. Microvesicles are occasionally observed fusing with these macrovesicles.

The largest macrovesicles are the type 2 macrovesicles, which are found throughout the mid-regions of the cell (Fig. 2B). These macrovesicles are more spherical in shape than the type 1 macrovesicles, and contain considerable quantities of "clumped" electron-dense material which, in some cases, appears to be membranous in nature. These macrovesicles are occasionally surrounded by a peripherally separated membrane, which gives this macrovesicle the appearance of having a double or triple membrane. The outer membrane of type 2 macrovesicles stain considerably darker than those of the type 1 macrovesicles.

Type 3 macrovesicles are similar to the type 2 macrovesicles in many respects (Fig. 2C). Both are spherical in shape and surrounded by a peripherally separated membrane. Also, both macrovesicles contain materials which often appear clumped. The major differences between the type 2 and type 3 macrovesicles are location, size, and electron opacity. Type 3 macrovesicles are located almost exclusively in the basal half of the cell. These macrovesicles are considerably smaller in size, and their contents are more electron dense than the type 2 macrovesicles.

The nucleus of the digestive cell is basally situated and surrounded by a double-layered nuclear envelope with several membrane pores (Fig. 2C). The nucleus contains conspicuous clumps of chromatin. Occasionally, a prominent, single nucleolus is present. Other organelles in this cell type include granular endoplasmic reticulum (GER), which is typically located adjacent and parallel to the lateral plasma membrane, and mitochondria, which are found throughout the cytoplasm.

Infrequently, the sub-apical region of digestive cells gives the appearance of sloughing off or shedding. During these occasions, the apical membrane no longer displays its characteristic microvilli or pinocytotic activity. Within the region apparently being shed, various microvesicles and macrovesicles are present.

The crypt of the digestive tubule of *C. virginica* consists of non-flagellated and flagellated basophil cells at an approximate proportion of 1:1. The non-flagellated basophil cells are pyramidal-shaped with the narrow end projecting toward the mouth of the crypt and the broad basal end resting on a thin basement membrane (Fig. 3A).

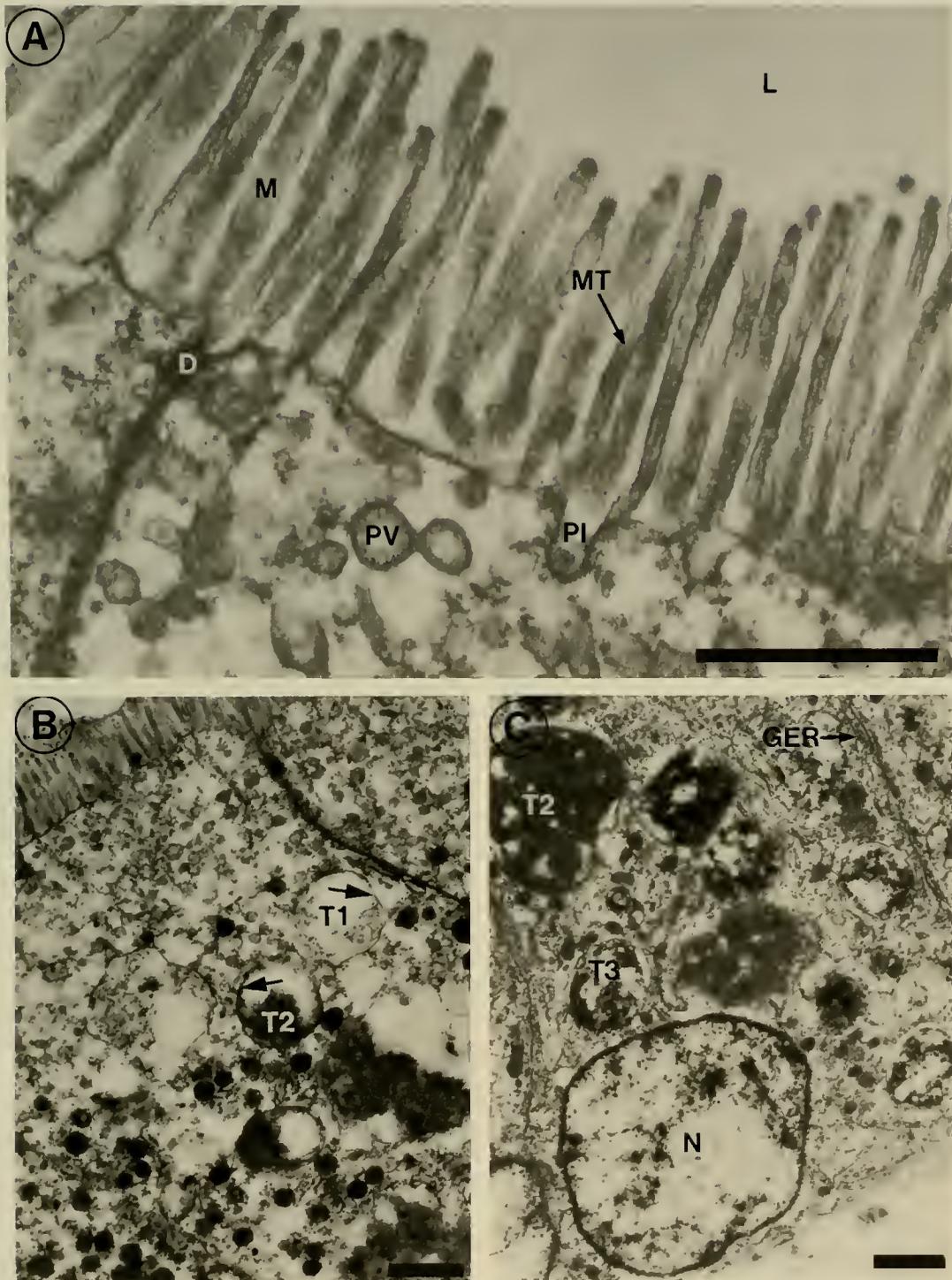


Figure 2. Digestive cell. All bars = 1.0  $\mu\text{m}$ . (A) High power electron micrograph featuring the apical portion of the oyster digestive cell. The most conspicuous feature of the apical plasma membrane are the well-developed microvilli (M) which extend into the lumen (L) of the tubule. Within the microvilli, microfilaments (MT) are evident. The presence of pinocytotic activity in the apical plasma membrane is suggested by the pinocytotic invagination (PI) and several pinocytotic vesicles (PV). D = desmosome (32,800 $\times$ ). (B) Apical half of the digestive cell showing type 1 and type 2 macrovesicles (T1 and T2, respectively). Note the differences in the bordering membranes of these two types of macrovesicles: the bordering membrane of the type 2 macrovesicle is considerably thicker, and stains denser than that of the type 1 macrovesicle (indicated by arrows) (10,000 $\times$ ). (C) Basal half of the digestive cell showing type 2 and type 3 macrovesicles (T2 and T3, respectively). Granular endoplasmic reticulum (GER) is found adjacent and parallel to the lateral plasma membranes. N = nucleus (10,000 $\times$ ).

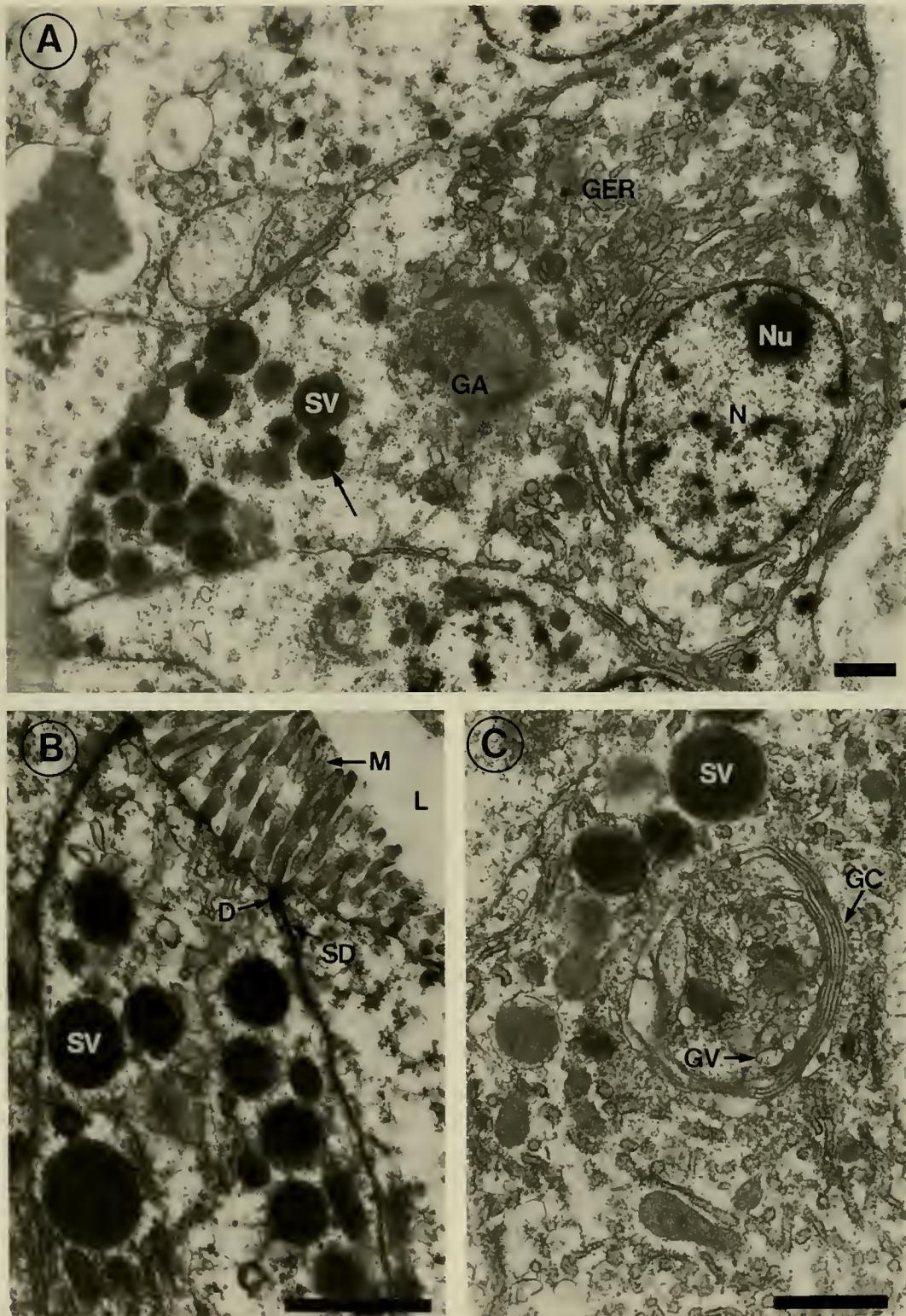


Figure 3. Non-flagellated basophil cell. All bars = 1.0  $\mu\text{m}$ . (A) Cross section through a non-flagellated basophil cell showing the conspicuous granular endoplasmic reticulum (GER), a well-developed Golgi apparatus (GA), and numerous secretory vesicles (SV). Note the presence of a globular core within several of the secretory vesicles (indicated by arrow). The basally situated nucleus (N) contains a conspicuous nucleolus (Nu) (9,300 $\times$ ). (B) Apical half of the non-flagellated basophil cell showing numerous microvilli (M) extending into the lumen (L). Adjacent cells are connected through a desmosome (D), septate desmosome (SD), and tight junctions (not evident in this micrograph) (21,000 $\times$ ). (C) High power electron micrograph featuring further detail of the Golgi apparatus. The essential components of the Golgi apparatus are the numerous, cup-shaped cisternae (GC) and the apparent formation of Golgi vesicles (GV) in the innermost cisterna. Secretory vesicles of various electron density are present in the region surrounding the Golgi apparatus (17,100 $\times$ ).

Non-flagellated basophil cells are characterized by their well-developed organelles. The most conspicuous of these is the GER, which occupies the basal half of these cells (Fig. 3A). The GER is continuous with the nuclear envelope, and both are generously studded with ribosomes. The basally situated nucleus contains a single, large nucleolus surrounded by peripherally located clumps of chromatin.

In the apical half of the non-flagellated basophil cell, several conspicuous membrane-bound secretory vesicles are present (Fig. 3A). Most of these vesicles display electron-dense contents of various densities. Occasionally, globular cores are observed within the center of these secretory vesicles. The apical plasma membrane contains several well-formed microvilli projecting into the lumen of the tubule (Fig. 3B). As in the digestive cells, the lateral plasma membranes of adjacent cells are linked sequentially through a desmosome, a septate desmosome, and a tight junction.

An extensive Golgi apparatus is found lying above the nucleus (Fig. 3C). The Golgi apparatus consists of several stacked, cup-shaped cisternae. The innermost cisternae frequently demonstrate an increasing distention of their peripheral regions, forming Golgi vesicles at their terminal ends; their contents are moderately electron dense, and similar in appearance to the contents of the cisternae. Microvesicles and macrovesicles of variable electron density can be found in the vicinity of the Golgi apparatus.

By contrast, the flagellated basophil cell shares few characteristics with the non-flagellated basophil cell. The general form of the flagellated basophil cell is columnar, although the lateral margins are slightly tapered toward the apical region of the cell (Fig. 4A). This cell type can be found throughout the crypt region

(adjacent to both digestive cells and non-flagellated basophil cells). Those features shared by both types of basophil cells include the microvillous apical plasma membrane and the basally situated nucleus. Numerous free ribosomes are also present throughout the cytoplasm of this cell.

Absent from the flagellated cell is the conspicuous organelles found in the non-flagellated basophil cell, such as the GER, Golgi apparatus, and secretory vesicles. Instead, the conspicuous feature of this cell type is the presence of a single, long flagellum (Fig. 4B). This flagellum demonstrates typical features including the "9 + 2" arrangement of microtubules, a basal body, and rootlet fibers. Occasionally, mitochondria are present adjacent to the rootlet fibers.

## DISCUSSION

Although the fine structure of the oyster digestive tubule is similar to that described for other bivalves (Sumner 1966, Pal 1971, 1972, Owen 1972, Huca et al. 1982), only the marine cockle, *C. edule* (Owen 1970) and the Indian estuarine bivalve, *M. meretrix* (Pal et al. 1990) possess the same three general cell types observed in the eastern oyster. These three cell types include the digestive cell, non-flagellated basophil cell, and flagellated basophil cell. In oysters, the tall, prismatic digestive cells are characterized by their extensive system of microvesicles and macrovesicles. The pyramidal, non-flagellated basophil cells have several well-developed organelles including a conspicuous Golgi apparatus and extensive GER. By contrast, the elongate, flagellated basophil cells lack the well-developed organelles of the non-flagellated cell type. Instead, these cells are characterized by a conspicuous, single flagellum. Both the flagellated and non-flagellated basophil cell types are located within the crypts of the oyster digestive tubule.

One particular feature appears to be unique to the fine structure of the oyster digestive tubule. Previous studies on bivalves have reported the presence of basally situated lipid droplets in both the digestive cells and basophil cells (Sumner 1966, Owen 1970, 1973, Pal 1971, 1972 Huca et al. 1982, Pal et al. 1990). In this study, no such lipid droplets were observed in these cells. Instead, small amounts of lipid were observed within the basement membrane surrounding the digestive tubules. Similar observations were reported in a histochemical study of the digestive tubules of the Portuguese oyster, *Crassostrea angulata* (Lamarck 1819) (Mathers 1973). The presence of lipid droplets in the basement membrane may be indicative of a long-term storage role for this tissue. Alternatively, the lipid may eventually be transported elsewhere, such as the hemolymph, and these droplets only represent the temporary storage of lipid. Nevertheless, these observations suggest that the connective tissue surrounding the digestive tubules do function in the storage of lipids in some members of the genus *Crassostrea*.

It is also interesting to note that previous studies on the digestive tubules of oysters collected from Canada (Shaw and Battle 1957) and New Jersey (Eble 1966) have described a winter, cold-torpor resting phase, in which the shape of digestive cells change from columnar to cuboidal. No such seasonal changes were observed at the ultrastructural level, or at the light microscopy level using morphometric techniques to quantify mean epithelial height (Weinstein 1994). In fact, oysters collected during January 1993, from Clambank Creek had significantly greater mean epithelial

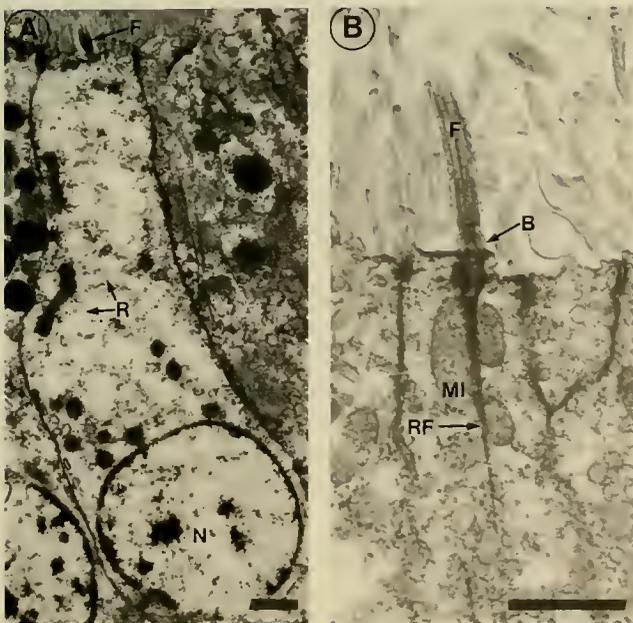


Figure 4. Flagellated basophil cell. All bars = 1.0  $\mu\text{m}$ . (A) Cross section through a flagellated basophil cell demonstrating the absence of well-developed organelles within the cytoplasm. Numerous free ribosomes (R) are present in the cytoplasm, and a single flagellum (F) is present at the apical plasma membrane. N = nucleus (9,000 $\times$ ). (B) High power electron micrograph featuring the flagellum of a flagellated basophil cell. The flagellar basal body (B) and rootlet fiber (RF) are evident in this micrograph. Note the presence of mitochondria (MI) adjacent to the rootlet fiber (22,500 $\times$ ).

thickness than during certain other seasons. Apparently, oysters from South Carolina do not undergo a cold-torpor phase during winter as described in oysters from more northern regions of its range.

The results of this study strongly support the long-held belief that digestive cells in oysters are specialized for the uptake and intracellular digestion of food materials (Yonge 1926, Shaw and Battle 1957). The electron micrographs presented here clearly demonstrate pinocytotic activity in the apical plasma membrane. These observations are consistent with earlier histochemical studies which found that the apical membrane of the oyster digestive cell stains intensely for alkaline phosphatase (Eble 1966). Alkaline phosphatase is indicative of cells involved in absorptive processes. Although the process of phagocytosis was not demonstrated in this study, it can not be excluded based on these results. The oysters examined in this study were collected at low tide; therefore, they were not actively feeding at the time of collection. However, feeding studies in the cockle (*C. edule*) suggest that phagocytosis is an important mechanism of intracellular digestion in bivalves (Owen 1970).

The various microvesicles and macrovesicles observed in the oyster digestive cells represent the lysosomal-vacuolar system described in other bivalves (Sumner 1966, Owen 1970, 1972, Pal 1972, Pal et al. 1990). The type 1 macrovesicles described here in oysters are probably phagosomes, or vacuoles within which the ingested material initially accumulates. This is supported by the frequently observed fusion between these macrovesicles and the pinocytotic vesicles. Similar observations have been previously reported in *C. edule* (Owen 1970).

The type 2 macrovesicles in oysters are probably lysosomes and phagolysosomes. Structurally similar macrovesicles in *C. edule* demonstrate intense activity for acid phosphatase, a lysosomal enzyme (Owen 1970). The presence of acid phosphatase has also been demonstrated in certain regions of the oyster digestive cell (Eble 1966). Based on those micrographs presented by Eble (1966), the regions of intense acid phosphatase staining generally coincide with those regions where the type 2 macrovesicles were located in this study.

The basally situated type 3 macrovesicles in oysters are similar in structure to the residual bodies described in *C. edule* (Owen 1970) and the nutclam, *Nucula sulcata* (Bronn 1831) (Owen 1973). Residual bodies are believed to contain indigestible food materials, and represent the end-point of intracellular digestion (de Dève and Wattiaux 1966). The mechanism for the elimination of the residual bodies was not obvious in this study; however, the "shedding" of the apical portion of the digestive cell was occasionally observed. Shedding has been proposed as a mechanism for the elimination of indigestible material in bivalves, and it has been previously observed in the marine clam, *Lasaea rubra* (Montagu 1803) (Morton 1956, McQuiston 1969); the edible oyster, *Ostrea edulis* (Linnaeus 1750) (Mathers 1972); the mahogany quahog, *Arctica islandica* (Linnaeus 1767) (Palmer 1979); and the freshwater mussel, *Diplodon delodontus* (Lamarck 1819) (Huca et al. 1982). Based on the observations reported here, shedding may represent one mechanism of elimination of indigestible materials in oysters.

The non-flagellated basophil cell in oysters contains all the essential components of a cell specialized for the production of extracellular enzymes. These features include a conspicuous nucleolus, an extensive GER, a well-developed Golgi apparatus, and several membrane-bound, secretory-type vesicles in the sub-apical

portion of the cell. Basophilic cells with similar ultrastructural features have been identified in *A. anatina* (Sumner 1966), *C. edule* (Owen 1970), and *M. meretrix* (Pal et al. 1990).

Despite all the ultrastructural evidence suggesting that these basophilic crypt cells are specialized for the production of enzymes (Sumner 1966; McQuiston 1969, Owen 1970, 1973, Pal 1971, Huca et al. 1982, Pal et al. 1990), considerable controversy exists concerning their ability to secrete these enzymes, which presumably would be used in extracellular digestion. This controversy is based upon evidence which contradicts the secretory nature of these cells. First, a micrograph showing the fusion of secretory vesicles with the cell membrane has yet to be published (Pal 1971, Pal et al. 1990). Moreover, histochemical studies on the digestive tubules of *A. islandica* demonstrated the lack of enzymatic activity within basophil cells (Payne 1979).

Such contradictory information led Pal (1971) to speculate that the majority of secretory vesicles in the basophil cells of the soft-shell clam, *Mya arenaria* (Linnaeus 1758) are autodigested before they can be extracellularly excreted. This conclusion was based upon the observation that numerous lysosome-like bodies were present in the sub-apical region of these cells (Pal 1971). Although similar observations have been made in other bivalves (Huca et al. 1982, Pal et al. 1990), no histochemical evidence has been provided to support this contention.

By contrast, much of this contradictory evidence has not been observed in oysters. Detailed examinations made in this study did not reveal any lysosome-like bodies within the non-flagellated basophil cells of oysters. These observations are consistent with previous histochemical studies in oysters. These basophilic crypt cells do not exhibit acid phosphatase activity (Eble 1966). Thus, the secretory vesicles in oysters are not likely to be autodigested in lysosomes prior to excretion. Furthermore, the crypt cells of two closely related species of oysters (*O. edulis* and *C. angulata*) are known to actively secrete arylamidase and esterase (Mathers 1973). Therefore, it appears that the non-flagellated basophil cells in *C. virginica* probably secrete at least some extracellular proteins into the lumen of the tubule.

The flagellated basophil cell in *C. virginica* strongly resembles that described in *A. anatina* (Sumner 1966), *C. edule* (Owen 1970), and *M. meretrix* (Pal et al. 1990). The general features of this cell type are consistent with that of an immature, undifferentiated stem cell. These features include the absence of several well-developed organelles including the Golgi apparatus and GER, and the presence of numerous free ribosomes in the cytoplasm. However, the lack of mitotic figures observed during this study cast doubt on the likelihood that these cells serve as a progenitor of the other digestive tubule cells in oysters.

Considerable controversy exists concerning the ability of the flagellated basophilic crypt cell in bivalves to serve as a stem cell. Following the earlier work of Yonge (1926), Sumner (1966) regarded these cells in the tubules of *A. anatina* as immature stem cells. Similar observations in *C. edule* led Owen (1970) to suggest that the flagellated basophil cell in this species was a precursor to both the non-flagellated, mature basophil cell and the digestive cell. Payne (1979) also came to similar conclusions following a histochemical study of *A. islandica*. In this study, Payne (1979) demonstrated the presence of only one basic cell type in the digestive tubule epithelium.

Recently, Pal et al. (1990) reported the absence of mitotic

figures in the basophilic crypt cells of *M. meretrix*, and concluded that the basophil cells in bivalves are unlikely to serve as progenitors of digestive cells. The observations reported here for oysters offers some support for this contention. Further research is needed to determine which cells, if any, act as the stem cell of digestive cells in bivalves.

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## DIGESTIVE TUBULE ATROPHY IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791), EXPOSED TO SALINITY AND STARVATION STRESS<sup>1</sup>

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**ABSTRACT** Oysters sampled in February, 1992 from a low salinity site (3 ppt) in Apalachicola Bay, FL showed digestive tubule atrophy when compared with oysters from a higher salinity site (18 ppt) 16 km away. Experiments designed to induce tubule atrophy in the laboratory consisted of two starvation and two salinity stress tests. To quantify tubule condition for each oyster, inside to outside tubule diameter ratios were calculated from 20 tubules per animal using an ocular micrometer. Higher tubule ratios indicated greater tubule atrophy. The experiments showed that poor nutrition, during low salinity, may have played a significant role in the tubule atrophy of Apalachicola Bay oysters.

**KEY WORDS:** Oysters, digestive diverticula, atrophy, salinity, starvation

### INTRODUCTION

The morphology and function of bivalve mollusc digestive tubules have been described for numerous species (Purchon 1968, Owen 1974, Langton 1975, Morton 1983). Most bivalves have a series of blind-ending tubules which join with the stomach. Typical tubule epithelia consist of two main cell types, digestive cells and basophils or crypt cells (Owen 1974). These cells are generally a tall, columnar, nonciliated epithelium but can change to simple cuboidal or squamous depending on the feeding phase of the animal (Owen 1974, Morton 1977, 1983, Robinson and Langton 1980).

Although the tubules of many different molluscan species have been studied, most research has focused on the mussel *Mytilus edulis*. Numerous studies of the effects of xenobiotic (Bayne 1976, Moore et al. 1978, Lowe et al. 1981, Rasmussen 1982, Rasmussen et al. 1983, 1985, Lowe 1988, Moore 1988, Auffret 1988) and environmental stressors (Thompson et al. 1974, Bayne et al. 1978, Moore 1982, Pipe and Moore 1985, Widdows and Johnson 1988) on *M. edulis* digestive diverticula have also been conducted. It appears that stress, regardless of source, causes epithelial atrophy or thinning and may be due to the formation of autolysosomes in tubule cells (Lowe et al. 1981, Moore et al. 1989). Similar epithelial atrophy has been observed in other subtidal species under natural conditions such as: *Lymnaea truncatula* (Moore and Halton 1973), *Mytilus californianus* (Thompson et al. 1978), *Mercenaria mercenaria* (Robinson and Langton 1980) and *Crassostrea virginica* (Lowe et al. 1972, Couch 1984, Weis et al. 1993).

During a monthly physiological survey of oysters (*C. virginica*) from two sites in Apalachicola Bay, FL, a freshet from the Apalachicola River occurred due to heavy rainfall in February of 1992. A large part of the bay was exposed to low salinity for almost 3 weeks. At the time of sampling, 2 weeks into the freshet, salinity was 3 parts per thousand (ppt) at one station (Dry Bar) and 18 ppt at another station (Cat Point), approximately 16 km away. Histological examination of oysters revealed that oysters from the low salinity site had digestive tubule atrophy whereas those from the higher salinity site did not. This was unusual since prior and

subsequent samples recorded no evidence of tubule atrophy at either station when salinities were above 10 ppt. It appeared that low salinity may have played a role in tubule condition in oysters at the Dry Bar site.

Digestive tubule atrophy has been associated in *M. edulis* with abrupt changes in salinity (Bayne et al. 1981, Pipe and Moore 1985). However, no studies have been made with *C. virginica* to determine if its digestive tubules behave in a similar manner. Thus, a series of laboratory experiments were performed in which salinity and nutrition were controlled in an attempt to induce similar effects (tubule atrophy).

### MATERIALS AND METHODS

Four experiments were conducted at the United States Environmental Protection Agency, Gulf Breeze Florida laboratory over a two year period.

#### Experiment 1

Experiment 1 tested the hypothesis that low salinity can cause tubule atrophy in oysters. Animals were collected from Apalachicola Bay, FL in March of 1992 and maintained in a control flow-through seawater aquarium at 20–22 ppt salinity and 21°C for one week. Twelve oysters were then processed for histology and 20 animals were removed from the control flow-through aquarium and placed into each of two aerated 185 L static aquaria at 22 ppt salinity. These aquaria were located in larger holding tanks where flow-through seawater from Santa Rosa Sound controlled their temperature. This assured that control flow-through and experimental aquaria would always be at the same temperature. This procedure was also employed in the next three experiments. Salinity for this and subsequent experiments was adjusted and maintained with Instant Ocean® sea salt. One static aquarium was maintained at 20–22 ppt throughout the test while the other was gradually diluted over a 48 hr period to 3–7 ppt with distilled water. After salinity reduction, oysters in static aquaria were fed two liters of algae, *Isochrysis* sp. ( $3-5 \times 10^5$  mL<sup>-1</sup> stock concentration) every third day. Water changes (50%) in the static aquaria were made every three days at test salinities. At the end of 8 and 15 days, 10 oysters from each aquarium were carefully sacrificed and a 1 cm transverse section through the midsection processed for histological examination. Sections were fixed in

\*Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

<sup>1</sup>Contribution No. 900, of the Gulf Breeze Environmental Research Laboratory.

Davidson's (Shaw and Battle 1957) solution for 48 hrs and then dehydrated and embedded in Paraplast® embedding medium. Tissues were sectioned at 7  $\mu\text{m}$ , stained with Harris' hematoxylin and eosin and examined with light microscopy.

Oyster digestive tubules were measured from histological sections using a light microscope (200 $\times$ ) equipped with an ocular micrometer. Histological cross sections were divided into 4 quadrants (Fig. 1). Five randomly selected tubules from each quadrant (total of 20) were measured and two sets of measurements, perpendicular to each other, were taken from each tubule. Internal (luminal) tubule diameters from both sets were summed and divided by the sum of the two external diameter measurements to obtain a "tubule" ratio. The shape of tubule epithelia varies, so measurement criteria were established for the different shapes encountered (Fig. 2). The average ratio for all 20 measurements was designated the oyster tubule ratio. Thus higher tubule ratios indicated greater tubule atrophy. Analysis of variance (ANOVA) was performed on individual oyster tubule ratios and differences between treatments and sampling times were determined using the Duncan grouping method ( $p \leq 0.05$ ) in this and subsequent experiments (Steel and Torrie 1960).

### Experiment 2

This experiment was initiated to determine the effect of starvation on oyster digestive tubules. Animals were collected from Apalachicola Bay on Aug. 2, 1992, and held in a control flow-through aquarium (23 ppt/26°C). After one week 12 oysters were processed for histological examination as before. Twenty oysters were also removed from the control flow-through aquarium and placed into each of 2 static aquaria (185 L) at 23 ppt and 26°C. The static aquaria were wrapped with dark plastic to keep light out and retard algal growth. Oysters in the static aquaria were not fed for the duration of the experiment and water changes were made every 3 days. On days 7 and 15, 10 oysters were taken from the 2 static aquaria and control flow-through aquarium for histological processing and examination. Digestive tubule ratios and ANOVA calculations were performed using the methods described in Experiment 1.

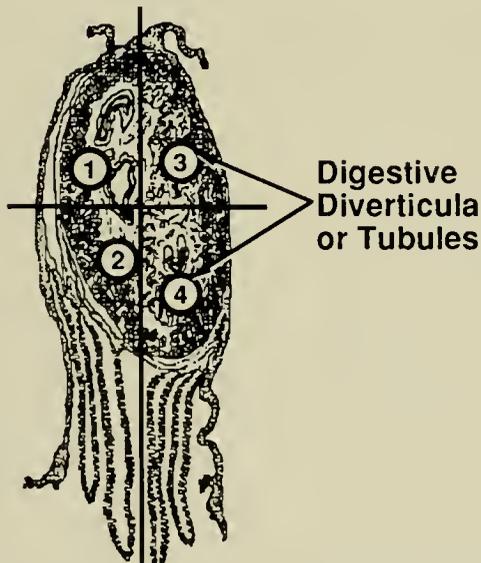


Figure 1. Cross section of oyster divided into quadrants.

## TUBULE TYPES

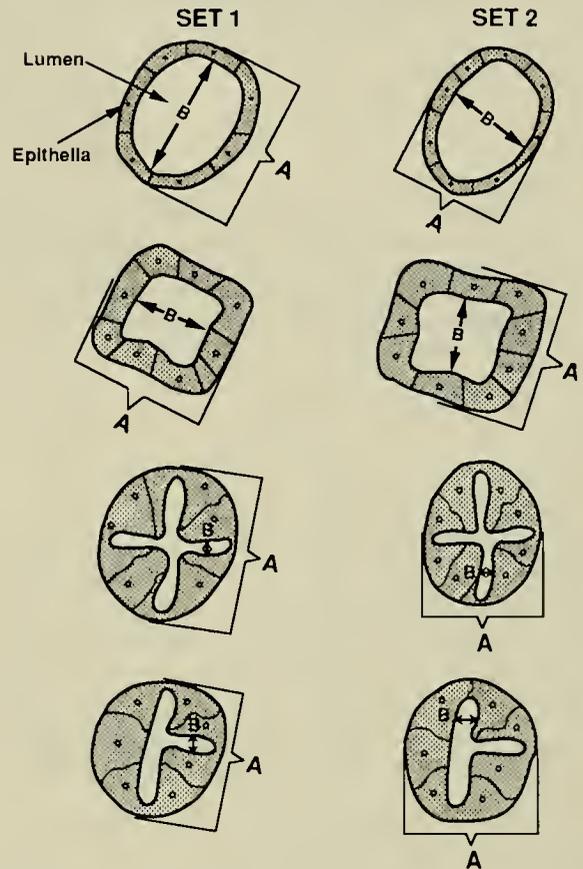


Figure 2. Morphological stages of digestive tubules showing where measurements are taken to give tubule ratios

$$\frac{B_1 \pm B_2}{A_1 \pm A_2} = \text{tubule ratio.}$$

### Experiment 3

Experiment 3 revisited the possible effect of salinity as in Experiment 1, but this time oysters were fed everyday. Oysters collected from Apalachicola Bay on December 1, 1992 were held in a control flow-through aquarium at 18–23 ppt salinity and 15–17°C. On day 0, 12 animals were removed and processed for histological examination as noted previously. Also, 36 oysters were removed from the control flow-through aquarium and placed into three, aerated, 185 L static aquaria at 22 ppt salinity. One static aquarium was maintained at 22 ppt salinity throughout the test while the salinity in the other two static aquaria was gradually reduced to 3–7 ppt or increased to 35–36 ppt over a 48 hr period. Temperature was kept at 15–17°C in all aquaria during the 21 day experiment and a 3 L mixture of algae (50% *Isochrysis* sp./50% *Chaetoceros* sp.,  $3\text{--}5 \times 10^5 \text{ mL}^{-1}$  stock concentration) was added to each static aquarium on a daily basis. Water changes (50%) in the static aquaria were made every three days with same salinity sea water. Twelve animals from each of the static and control flow-through aquaria were sacrificed on days 7, 14 and 21 for histological examination and digestive tubule measurement.

#### Experiment 4

The fourth experiment (December 1993) examined oyster tubule response to starvation and feeding. Animals were collected from Apalachicola Bay and held in a control flow-through aquarium for one week at 28 ppt salinity and 16°C. Forty-two oysters from the control flow-through aquarium were placed in each of two 185 L aerated aquaria. The aquaria were maintained at  $28 \pm 2$  ppt salinity for 22 days and 16°C for the first six days. On day 7 a cold front lowered water temperature to 14°C by day 8, 11°C by day 16 and 9°C by day 18. Temperature began to warm up several days later and by day 22 water was at 11°C. Experimental oysters were not fed for 16 days and the static aquaria were covered with black plastic to keep out light and minimize algal growth. Temperature, salinity and condition of oysters were monitored daily with water changes (50%) in the static aquaria completed every 3 days. On day 16 the plastic was removed and the starved animals were fed 4 liters of an algal cocktail (50% *Isochrysis* sp./50% *Chaocerosus* sp.,  $3-5 \times 10^5$  mL<sup>-1</sup> stock concentration) per tank. Feeding at this level was continued daily for another 6 days. Tissue sections from six oysters from each aquarium were processed for histological examination on days 2, 4, 8, 16, 18 and 22 and digestive tubule ratios were determined as in Experiment 1.

#### RESULTS

Experiment 1 tubule ratios, on day 0, averaged  $0.192 \pm 0.021$ . Histology of the oysters showed typical prespawm animals with tall columnar digestive tubule epithelia (Fig. 3) relative to oysters with squamous epithelia (Fig. 4). On day 3, oysters in each of the two static aquaria were fed 2 liters of *Isochrysis* sp. After 2 hrs, oysters

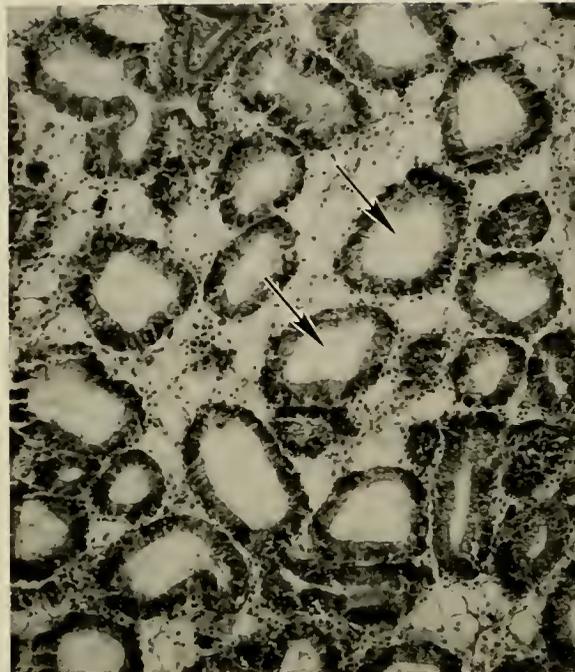


Figure 4. Atrophied digestive tubules of oyster. Note wide lumens (arrows) and squamous to cuboidal epithelia of tubules.

in the static moderate salinity (22 ppt) aquarium had cleared the algae whereas the static low salinity (3-7 ppt) aquarium remained cloudy for 2 more hours. However, at day 6 when oysters were fed, both aquaria cleared after 2 hrs.

The different experimental conditions in Experiment 1 elicited 3 different responses. First, those oysters maintained in flow-through seawater had low tubule ratios throughout the experiment whereas those in the static aquaria exhibited higher ratios (Table 1, Fig. 5A). Secondly, tubule ratios for oysters in the static aquarium held at 20-22 ppt salinity increased to  $0.459 \pm 0.078$  by day 8, then leveled. Finally, tubule ratios in the static aquarium at 3-7 ppt salinity increased to  $.594 \pm 0.042$  by day 8 to  $.690 \pm 0.012$  on day 15 (Table 1, Fig. 5A) and Duncan grouping showed a significant difference among all aquaria.

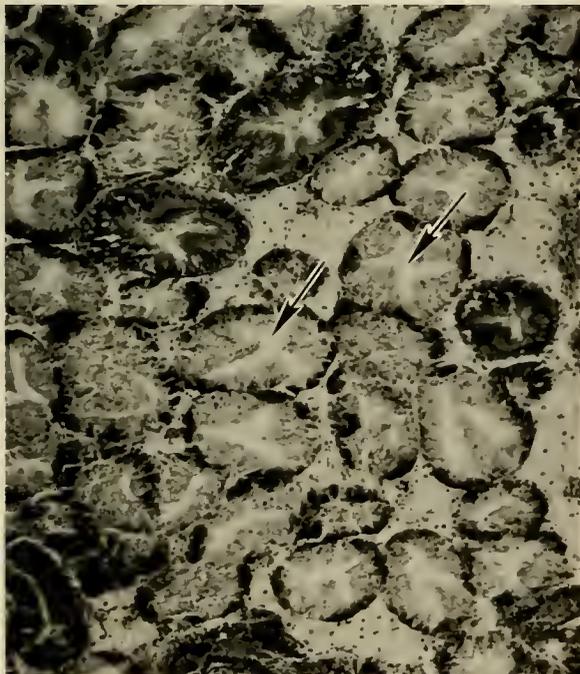


Figure 3. Digestive tubules of oyster. Note narrow lumens (arrows) and columnar epithelia of tubules.

TABLE 1.

Experiment 1. Effects of low salinity on oyster tubules while fed partial ration.

Day	Aquarium	Tubule Ratios (SE)	Duncan
0	FT-(22 ppt/21°C)	$\bar{x}$ 0.192 (0.021)	
8	FT-(22 ppt/21°C)	$\bar{x}$ 0.168 (0.033)	B
	1-(7 ppt/21°C)	$\bar{x}$ 0.594 (0.042)	A
	2-(20 ppt/21°C)	$\bar{x}$ 0.459 (0.078)	A
15	FT-(20 ppt/21°C)	$\bar{x}$ 0.233 (0.038)	C
	1-(3 ppt/21°C)	$\bar{x}$ 0.690 (0.012)	A
	2-(22 ppt/21°C)	$\bar{x}$ 0.425 (0.077)	B

Flow-through aquaria received continuous ration of plankton. Aquaria 1 and 2 were static and fed *Isochrysis* sp. ( $3-5 \times 10^5$  ml<sup>-1</sup> stock concentration) every third day. (FT = Flow-through control).

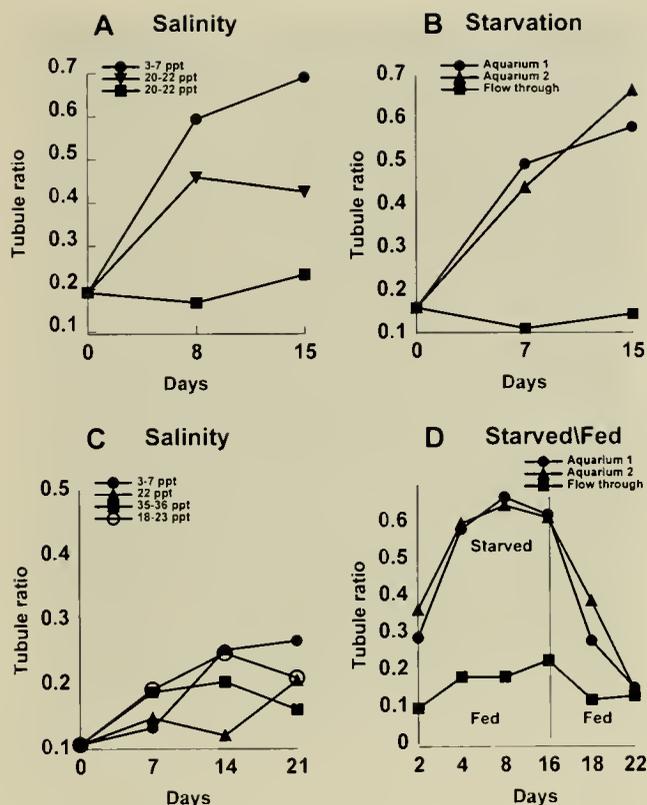


Figure 5. Digestive tubule ratios from oysters held in static and flow-through aquaria and exposed to salinity and starvation stresses. (A) Experiment 1—Salinity stress with incomplete ration (2 L *Isochrysis* sp. every 3 days to both static aquaria). ● Salinity lowered from 20 ppt to 3–7 ppt over two days and held in static conditions. ▼ Salinity kept at 20 ppt in static conditions. ■ Flow-through aquarium with raw seawater. Tubule ratios after 15 days were significantly different in all aquaria. Temperature = 21°C. (B) Experiment 2—Starvation stress with no food given to each of two replicate static aquaria vs a flow-through aquarium with raw sea water. Tubule ratios after 15 days showed no significant differences between static aquaria but static aquaria were significantly different than the flow-through aquarium. Temperature = 26°C. (C) Experiment 3—Salinity stress with full rations (3 L 50% *Isochrysis* sp./50% *Chaetoceros* sp. every day to three static aquaria). ● Salinity lowered from 23 ppt to 3–7 ppt over two days and maintained static. ▲ Salinity adjusted to 22 ppt and maintained static. ■ Salinity raised from 23 ppt to 35–36 ppt over two days and kept static. Flow-through aquarium with flow-through raw sea water. Tubule ratios at all sampling dates show no significant differences among all tanks. Temperature = 15–17°C. (D) Experiment 4—Oysters were starved for 16 days then fed full ration (4 L 50% *Isochrysis* sp./50% *Chaetoceros* sp. to both static aquaria) every day for another 6 days. Tubule ratios increase significantly in starved oysters but decreased with renewed feeding to values similar to those in the flow-through aquarium. Temperature = 16–9°C.

Oysters in Experiment 2 (starvation) began with low tubule ratios of  $0.161 \pm 0.046$  (Table 2). After 7 days, ratios in oysters from the flow-through aquarium were still low whereas ratios were much greater in animals from the two unfed static aquaria (Table 2, Fig. 5B). At the end of 15 days the tubule ratios remained low in the flow-through aquarium but had increased considerably in the two static aquaria (Table 2, Fig. 5B). Analysis of variance showed unfed oyster tubule ratios to be significantly higher at days 7 and

TABLE 2.

Experiment 2. Effects of starvation on oyster digestive tubules.

Day	Aquarium	Tubule Ratios (SE)	Duncan
0	FT-(23 ppt/26°C)	0.161 (0.046)	
7	FT-(23 ppt/26°C)	0.112 (0.002)	B
	1-(23 ppt/26°C)	0.494 (0.091)	A
	2 (23 ppt/26°C)	0.440 (0.080)	A
15	FT-(23 ppt/26°C)	0.146 (0.009)	B
	1-(23 ppt/26°C)	0.580 (0.053)	A
	2-(23 ppt/26°C)	0.665 (0.009)	A

Flow-through aquaria receive continuous ration of plankton. Aquaria 1 and 2 static with no ration for duration of experiment. (FT = Flow-through control).

15 than those of animals held in the flow-through aquarium. Also, there were no significant differences between unfed oysters at either day 7 or day 15 (Fig. 5B).

Oysters in Experiment 3 (salinity) began with average tubule ratios of  $0.107 \pm 0.018$  and the ratios remained relatively low during the entire experiment. On day 7, tubule ratios ranged from  $0.133 \pm 0.024$  in the lowest salinity aquarium to  $0.192 \pm 0.054$  in the flow-through aquarium (Table 3, Fig. 5C). There was a slight increase in tubule ratios over the next 2 weeks with the flow-through and low salinity aquaria having the highest ratios (Table 3, Fig. 5C). However, there was no significant difference in the tubule ratios of oysters from all aquaria at specific sampling dates (Table 3, Fig. 5C).

Oysters sampled on day 2 in Experiment 4 (starvation) had tubule ratios of  $0.097 \pm 0.002$  for flow-through conditions (Table 4, Fig. 5D). In contrast, oysters in the two replicate experimental aquaria began to show higher values (Table 4, Fig. 5D). Analysis of variance showed that tubule ratios in oysters from flow-through conditions and aquarium 1 were not significantly different on day 2. However, there was a significant difference between oysters in flow-through conditions and those in aquarium 2. The tubule ratios

TABLE 3.

Experiment 3. Effects of salinity on oyster tubules while fed full ration.

Day	Aquarium	Tubule Ratios (SE)	Duncan
0	FT-(22 ppt/15°C)	0.107 (0.018)	
7	FT-(18 ppt/16°C)	0.192 (0.054)	A
	1-(3 ppt/16°C)	0.133 (0.024)	A
	2-(20 ppt/16°C)	0.148 (0.030)	A
	3-(35 ppt/16°C)	0.188 (0.047)	A
14	FT-(20 ppt/17°C)	0.248 (0.043)	A
	1-(7 ppt/17°C)	0.253 (0.079)	A
	2-(21 ppt/17°C)	0.121 (0.036)	A
	3-(36 ppt/17°C)	0.204 (0.057)	A
21	FT-(23 ppt/16°C)	0.210 (0.060)	A
	1-(6 ppt/16°C)	0.267 (0.054)	A
	2-(23 ppt/16°C)	0.206 (0.025)	A
	3-(36 ppt/16°C)	0.161 (0.024)	A

Flow-through aquaria receive continuous ration of plankton. Aquaria 1, 2 and 3 were static and fed full ration (3 L 50% *Isochrysis* sp./50% *Chaetoceros* sp.,  $305 \times 10^5$  mL<sup>-1</sup> stock concentration) daily. (FT = Flow-through control).

TABLE 4.

Experiment 4. Effects of starvation (15 days no food with full ration feeding at day 16) on oyster digestive tubules.

Day	Aquarium	Tubule Ratios (SE)	Duncan
2	FT-(28 ppt/16°C)	0.097 (0.002)	B
	1-(28 ppt/16°C)	0.287 (0.089)	A,B
	2-(27 ppt/16°C)	0.362 (0.115)	A
4	FT-(29 ppt/16°C)	0.181 (0.043)	B
	1-(27 ppt/16°C)	0.581 (0.077)	A
	2-(27 ppt/16°C)	0.596 (0.091)	A
8	FT-(24 ppt/14°C)	0.181 (0.052)	B
	1-(28 ppt/14°C)	0.667 (0.065)	A
	2-(28 ppt/14°C)	0.645 (0.094)	A
16	FT-(26 ppt/11°C)	0.225 (0.030)	B
	1-(27 ppt/11°C)	0.620 (0.091)	A
	2-(27 ppt/11°C)	0.612 (0.091)	A
18	FT-(27 ppt/9°C)	0.119 (0.004)	B
	1-(30 ppt/9°C)	0.278 (0.087)	A,B
	2-(30 ppt/9°C)	0.386 (0.097)	A
22	FT-(27 ppt/11°C)	0.129 (0.006)	A
	1-(29 ppt/11°C)	0.151 (0.029)	A
	2-(29 ppt/11°C)	0.146 (0.025)	A

Flow-through aquaria receive continuous ration of plankton. Aquaria 1 and 2 were static with no ration for 15 days and fed full ration (4 L 50% *Isochrysis* sp./50% *Chaetoceros* sp.  $3-5 \times 10^5 \text{ mL}^{-1}$ ) daily for the next 6 days. (FT = Flow-through control).

continued to increase significantly in both experimental aquaria over the next 6 days, reaching peak tubule ratios of  $0.667 \pm 0.065$  and  $0.645 \pm 0.091$  whereas the tubule ratios did not significantly change in the flow-through aquarium (Table 4, Fig. 5D). Analysis of variance detected significant differences between experimental

and flow-through aquaria and no significant difference between the two experimental aquaria. By day 18, two days after feeding was initiated, average tubule ratios in oysters from the experimental aquaria had dropped and digestive tubule ratios for oysters in flow-through still remained low (Table 4, Fig. 5D). Analysis of variance (day 18) showed only one experimental aquarium with tubule ratios significantly different from that of oysters in the flow-through aquarium. At day 22 tubule ratios in oysters from the experimental aquaria were nearly the same as the tubule ratios in oysters from the flow-through aquarium (Table 4, Fig. 5D). Analysis of variance at this time showed no significant differences in tubule ratios among all aquaria.

## DISCUSSION

The results of these experiments indicate that nutritional stress probably played a significant role in the digestive tubule atrophy observed in Apalachicola Bay oysters. Tubule atrophy is not an unusual occurrence in bivalves (Morton 1983). The digestive tubules of most bivalves go through 4 morphological phases related to feeding and tidal rhythm (Owen 1966, Langton 1975, Robinson and Langton 1980, Morton 1983). These phases include a holding phase, an absorptive phase, a disintegrating phase and a reconstituting phase. In the first 2 phases, digestive cells of the tubules are columnar with a narrow lumen and, if the tubule ratio described here is applied, would have low tubule ratios. The third and fourth phase tubule cells are squamous to cuboidal with a wider tubule lumen than in the first two phases and would have higher tubule ratios.

Numerous studies have shown that, for intertidal bivalves such as *Cardiurn edule* (Morton 1970a, Owen 1970), *Macoma balthica* (Morton 1970b), *Geloina proxima* (Morton 1975) and *Venerupis decussata* (Mathers et al. 1979), feeding is discontinuous and related to tidal rhythms. This means that while the animals are

TABLE 5.

Summary of experimental parameters.

	Experiment			
	1	2	3	4
Salinity (ppt)	3-7-Static 1 20-22-Static 2	23-Static 1 23-Static 2	3-7-Static 1 20-23-Static 2 35-36-Static 3	27-30-Static 1 27-30-Static 2
Temperature (°C)	20-22-Flow-through Control 21	23-Flow-through Control 26	18-23-Flow-through Control 15 → 17	24-29-Flow-through Control 16 → 9 → 11
Ration	Statics 2 L of <i>Isochrysis</i> sp. every third day. Flow-through Normal plankton every day	Statics none Flow-through Normal plankton every day	Statics 3 L of 50% <i>Isochrysis</i> sp./50% <i>Chaetoceros</i> sp. every day. Flow-through Normal plankton every day	Statics no ration first 15 days, 4 L of 50% <i>Isochrysis</i> sp./50% <i>Chaetoceros</i> sp. every day for next 6 days. Flow-through Normal plankton every day
Stock Concentration	$3-5 \times 10^5 \text{ mL}^{-1}$	N/A	$3-5 \times 10^5 \text{ mL}^{-1}$	$3-5 \times 10^5 \text{ mL}^{-1}$
Length of Experiment (days)	15	15	21	22

feeding their digestive tubules are mostly in the first 2 phases of digestion with columnar epithelia and narrow lumens. When the tide recedes the majority of tubules revert into the last 2 phases of digestion with thin epithelia and wide lumens. However, in mid and subtidal bivalves, the first two phases are present the majority of the time because feeding is a relatively continuous process due to food availability (Robinson and Langton 1980). In *M. edulis*, phase 1 and 2 tubules comprise almost 90% of the morphological types (Langton 1975) and the same observations have been made in subtidal populations of *M. mercenaria* (Robinson and Langton 1980), *Pectin maxinius* (Mathers 1976), *Chiarnys varia* (Mathers et al. 1979) and *Ostrea edulis* (Wilson and LaTouche 1978).

Since *C. virginica* were subtidal at the sampling times, the tubule morphology and low tubule ratios observed in oysters held in the flow-through aquaria probably represented the normal unstressed state for these animals. The high tubule ratios observed in oysters sampled from Apalachicola Bay in February 1992 and experimental aquaria would indicate the animals were under some form of stress.

Previous investigators have shown that environmental pollutants can cause tubule atrophy in bivalves (Lowe et al. 1972, Bayne 1976, Moore et al. 1978, Lowe et al. 1981, Couch 1984, 1985, Lowe 1988, Moore 1988). However, since Apalachicola Bay is a relatively pristine estuary and there was no atrophy prior to or after the low-salinity event, the influence of xenobiotics was probably minimal.

Because the salinity at Dry Bar was low compared to Cat Point, it was originally hypothesized that low salinity may have been responsible for the high tubule ratios. Most bivalves react to salinity changes via intracellular volume regulation (Bayne 1976, Bishop 1976, Bayne et al. 1981, Moore et al. 1989). In low salinity situations many molluscs reduce intracellular amino acids concomitant with alterations in nitrogen metabolism and excretion (Bishop 1976). Also, the digestive cells of *M. edulis* play an important role in their ability to deal with abrupt increases in salinity by undergoing autophagy to supply a source of free amino acids (Bayne et al. 1981, Moore et al. 1989).

Studies have shown that when oysters were exposed to prolonged low salinity periods, both the rate of water transport and the time they remained open were decreased and, under extreme conditions, feeding and respiration ceased (Galtsoff 1964). The fact that oysters from the low salinity aquarium in Experiment 1 cleared the algae as fast as the static ambient aquarium by day 6 indicates that salinities in the 3–5 ppt range had little effect on the oysters ability to open and feed. Also, experiment 3 showed no effect of salinity. Oyster populations in Mobile Bay, AL have been observed to survive long periods of exposure to salinities ranging from 2–5 ppt (May 1972). Significant mortalities do occur when salinity remains below 2 ppt for long periods (Galtsoff 1964, May 1972).

Experiment 1 did not refute the hypothesis that salinity was a factor in digestive tubule atrophy. However, the fact that those oysters held at ambient salinity also had a significant tubule ratio increase indicated that some other factor was involved.

Experiment 2 revealed that poor nutrition may have been that other factor. Low digestive gland index and tubule degeneration have been reported in *M. edulis* starved for 4 weeks (Thompson et al. 1974) and in *M. californianus* digestive tubule epithelium was reduced in thickness after 13 days of starvation (Thompson et al. 1978). In contrast, *M. mercenaria* starved for 57 days showed little difference in tubule ratios between fed and unfed groups and

the proportion of Type II tubules (absorptive) actually increased (Robinson 1983). It was hypothesized that the combined effect of low metabolic rate, due to the starvation itself, and cropped bacteria from the water could have helped increase the number of Type II tubules.

Experiment 3 (Table 3, Fig. 5C) was designed to minimize the effects of starvation. The low tubule ratios and lack of significance among different salinity groups indicate that nutrition, rather than salinity, is the primary cause for digestive tubule ratios in oysters increasing to high levels. It is interesting that the tubule ratios of high-salinity oysters had the lowest ratios after 21 days. In contrast, *M. edulis* exposed to abrupt increases in salinity showed considerable tubule thinning due to fusion of lysosomal vacuoles into giant lysosomes which resulted in autophagy (Pipe and Moore 1985).

Experiment 4 was performed to confirm the role of nutrition in tubule atrophy and to determine the relative response times of oyster tubules to starvation and feeding. Even though the water temperature was low (average 12°C) the tubule's ability to respond rapidly to re-feeding after starvation for 15 days is similar to other bivalves. Studies with *Crassostrea gigas* (Morton 1977) and *O. edulis* (Wilson and LaTouche 1978) showed changes in digestive tubule morphology could occur in less than 12 hours. These changes, as already discussed, depend on food availability and are normal responses for intertidal bivalves (Langton and Gabbott 1974, Robinson and Langton 1980). Also, *M. edulis* and *C. edule* had a significant increase in tubule atrophy when kept out of water for up to 96 hrs; the tubules reverted to a columnar morphology within 24 hrs of resubmergence (Moore et al. 1979).

Because temperature in all aquaria was controlled by flow-through seawater from the outside environment, temperature varied between different experiments. Also, in Experiments 3 and 4, temperature varied during individual tests. These temperature differences may have influenced the rate of response but did not appear to affect overall tubule response. In Experiments 1, 2 and 4 oyster tubules responded alike to poor nutrition even though there was a large variation in temperatures. Also, all experiments showed that as long as control flow-through and experimental oysters received a proper ration, tubule ratios remained or became low regardless of temperatures.

It is likely that poor nutrition played a role in the tubule atrophy in *C. virginica* from the Dry Bar site in Apalachicola Bay. The low salinity measured there could have been an indicator of a changing water mass that caused a change in types and/or amounts of food the oysters regularly ingest or caused them to stop feeding. Nutritive stress would have stopped when salinity and/or food availability returned to normal levels. In fact, tubule ratios were again low in the next sampling period. The rapid change in tubule ratios is important to consider if oyster tubule morphology is being used as a bioindicator of pollution. While tubule ratios may be useful as a measure of contaminant exposure, many natural environmental conditions, including nutrition, salinity, spawning or tides may cause oyster diverticula to change rapidly and as others have pointed out (Weis et al. 1993, Couch 1993), it may be a general (non-specific) indicator of stress in the animal.

#### ACKNOWLEDGMENT

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## ECONOMIC ANALYSIS OF THE POTENTIAL FOR EASTERN OYSTER (*CRASSOSTREA VIRGINICA* GMELIN, 1791) DEPURATION IN COASTAL NORTHWEST FLORIDA\*

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**ABSTRACT** Illness associated with the consumption of raw shellfish is one possible factor that has depressed demand for oysters *Crassostrea virginica* in coastal counties of northwest Florida. Controlled purification (deuration) has been identified as one method of improving public confidence in oysters and increasing sales. This economic analysis determines the anticipated costs of deuration processing for 12 design options with operating capacities from 30 to 498 bushels per week. The area of study is Dixie and Levy counties and adjacent water located in northwest Florida. Combined, these two counties have supplied approximately 15% of oyster landings for the state over the past 10 years. Based on the expected volume and cost of shellstock in the area, projected capital and operating costs, and the expected premium for deurated product, deuration is not an economically feasible method of shellfish processing in the area of study.

**KEY WORDS:** *Crassostrea virginica*, deuration, economics, shellfish processing

### INTRODUCTION

The majority of seafood-related illnesses in the U.S. are associated with the consumption of raw bivalve molluscan shellfish (National Institutes of Health (NIH) 1991). The disproportionate incidence of illness is largely related to the feeding behavior of molluscs and the fact that they are often eaten raw or partially cooked. Improper post-harvest handling can allow multiplication of bacteria (Reily et al. 1985, Cook and Ruple 1989), increasing the risk of illness.

Deuration can reduce the number of some illness-causing agents found in oysters, and has been identified as one way to increase public confidence and sales of oysters. The deuration process involves placing shellfish in tanks of decontaminated, flowing seawater for a minimum of 48 hours (National Shellfish Sanitation Program (NSSP) 1993b). At the end of 48 hours of active pumping, the animal is rid of gut contents and the pathogens therein. Water passing over the mantle and gills can carry away pathogens weakly attached to these tissues. Pathogens may also be reduced by natural die-off. At the same time some bacteria continue to multiply. The goal of deuration is to generate a net reduction in pathogens to a "safe" level, which is defined as a fecal coliform value of less than 20 per 100 grams of shellfish meat (NSSP 1993b). This compares to a fecal coliform standard of not greater than 230 per 100 grams meat for all other oysters sold at the wholesale level (Florida Administrative Code 1993).

Any increase in demand for oysters as a result of increased consumer confidence could economically benefit oyster harvesters, shellfish packing plants, and other businesses related to the

shellfish industry. The costs of deuration could be recovered by either a premium charge for deurated product, or the difference in shellstock costs between oysters harvested for direct-to-market sales and those that will undergo the intermediate step of deuration processing. This paper provides background on the regulatory environment for deuration facilities, determines the capital and operating costs for hypothetical facilities of different production capacities less than 480 bushels per week, and evaluates the feasibility of deuration processing for the study area based on cost and likelihood of recovering this cost. Cost calculations were made in a computer spreadsheet designed by the authors. The area of study is Dixie and Levy counties and adjacent water located in northwest Florida. Combined, these two counties have supplied approximately 15% of oyster landings for the state over the past 10 years.

### BACKGROUND AND REGULATORY ENVIRONMENT

The State Shellfish Control Agency (SSCA) in each state attempts to control the risk to human health of shellfish-borne pathogens by regulating the waters from which molluscs may be harvested. Surveys are designed and executed to identify sources of pollution that can impact growing areas. Of primary concern is contamination of fecal origin. Fecal coliform indicators, as part of a comprehensive sanitary survey, have been used to successfully eliminate major outbreaks of gastroenteritis caused by salmonella. A major weakness is that fecal coliforms do not appear to be an adequate indicator of human health risk from viral pathogens (Regan et al. 1993), nor risk from naturally occurring bacterial pathogens such as *Vibrio* (NIH, 1991).

Based on survey findings, coastal waters are designated as belonging to one of the six classifications of shellfish growing areas (Florida Department of Environmental Protection (DEP) 1993). Shellfish harvesting for direct marketing is permitted from approved (AP) or conditionally approved (CA) areas. Shellfish harvested from restricted (RE) and conditionally restricted (CR)

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areas must undergo controlled purification. (Shellfish may not be harvested from prohibited or unclassified areas). AP and RE waters close only under extraordinary circumstances such as storms and the presence of phytoplankton blooms. Periodic closure of CA and CR waters is governed by state management plans unique to each harvest area. In the area of study, all harvest waters are also closed during the months June through August.

With the satisfaction of certain provisions, oysters may be harvested from RE and CR waters and depurated prior to marketing. Based on Florida DEP resource assessment surveys, landings could increase by approximately 5,800 bushels (a standard bushel is equal to 60 pounds) per year in the study area if resource from RE and CR waters were utilized. This represents a 22% increase over the average annual harvests in the area of study from 1990 to 1992 (Florida DEP 1993). An additional 7,800 bushels are potentially available, but their location in harvest areas where suspected septic tank leakages have occurred has led to imposition of a lab analysis requirement to verify the absence of salmonella in any oysters that might be harvested for depuration. (This area will be referred to as the "Suwanee Sound" because the restricted area corresponds to waters in the Suwanee Sound area harvest map as defined by Florida DEP). The time delay caused by lab analysis and the short shelf life of harvested oysters discounts the use of this resource at the present time. However, facilities of larger size that could accommodate harvests from the Suwanee Sound are considered in this analysis.

Based on interviews with oyster harvesters regarding the costs of harvesting, harvesters would accept a 25% lower price for oysters harvested from CR and RE waters in the study area than they currently accept for oysters from AP and CA waters. This reduction in exvessel price results from a smaller fixed cost per harvested bushel due to a greater number of bushels harvested per trip. After adding the costs of a certified monitor who must accompany each boat or group of boats when oysters from CR and RE areas are harvested, the estimated maximum amount available for covering the costs of depuration is \$1.80 per bushel, or \$0.0064 per oyster, using an average of 280 oysters per 60 pound bushel.

A sales premium on depurated oysters could also cover the costs of depuration. While a recent study by Degner and Petrone (1994) found a willingness by some consumers to pay a premium for depurated oysters, this willingness was not reflected by restaurant managers who were also surveyed. The degree of liability associated with serving raw shellfish appears to be the major concern. Since most seafood in the U.S. is consumed away from home (O'Dierno and Gall 1994), it remains unclear as to whether there would exist a demand or price premium for depurated oysters.

Regulations regarding the design and operation of depuration facilities are found in the Florida Administrative Code (FAC) (1993) and are largely adopted from the National Shellfish Sanitation Program (NSSP) Manual of Operations. The NSSP is administered by the Interstate Shellfish Sanitation Conference (ISSC), which is a cooperative association between the individual states, the Environmental Protection Agency (EPA), Food and Drug Administration (FDA), the National Marine Fisheries Service (NMFS), and the shellfish industry. The NSSP Manual serves as a guide for the preparation of state shellfish regulations for shellfish harvesting and processing (NSSP 1993a, NSSP 1993b). Following is a summary of the most important considerations and implication of the NSSP manuals.

#### *Depuration Cycle Length*

Shellfish must be depurated for a minimum of 48 hours.

#### *Source of Process Water*

Process water for depuration facilities must be disinfected to meet biological standards of drinking water, and can not originate from waters classified as "prohibited." Irradiation is the only method of water disinfection that is currently sanctioned by the FDA (FAC 1993). Ozone and chlorination are alternative methods of disinfection used in European and Australian depuration facilities. While ozone use in the U.S. is not "illegal," at present it is considered a food additive and thus subject to costly regulation (McNamara 1991).

#### *Source of Shellfish*

A depuration facility is certified to depurate a particular shellfish product from a particular geographic area. Based on the type and source of the shellfish, regulators decide on the minimum time period required for depurating the product. This would be a minimum of 48 hours, but could be longer. A facility can be certified to depurate shellfish from several different areas, and may have different operating procedures for each (Mark Collins, pers. comm.-Florida DEP, Tallahassee, FL 1994).

#### *Water Volume and Water Flow per Bushel of Shellfish*

Regulations do not specify tank size or the number of shellfish per tank, but the NSSP recommends eight cubic feet of water per bushel of shellfish. A water flow of one gallon per minute (gpm) per bushel is required.

#### *Water Quality in the Process Tanks*

Water temperature and salinity must be "suitable" for depuration, meaning that water quality parameters should not be so different from harvest waters that shellfish pumping, and thus the effectiveness of depuration, would be lessened. Turbidity must be less than 20 JTU (Jackson Turbidity Units) and dissolved oxygen must be no less than 5 milligrams per liter. Tank waters must be tested for total coliforms every 24 hours (at 0, 24 and 48 hours for a 48 hour cycle), and this analysis must be performed by a state certified lab.

Although not specifically stated, regulators generally require that water from differing batches of depurated product (product that began the depuration cycle at different times) not be commingled, and that tank waters be discarded at the end of each cycle.

#### *Shellfish Meat Quality*

Meat samples are taken every 24 hours (at 0, 24, and 48 hours for a 48 hour cycle) and tested for fecal coliforms. The lab analysis from a state certified lab must demonstrate a fecal coliform count of less than 20 per 100 grams of meat before the shellfish may leave the facility. Product from each depuration unit is tested separately.

Regulations regarding effluent discharge and land easement are particular to each state and dependent upon the specific site location. This analysis assumes a zero-cost general permit for discharge and a \$1000 charge for the wetland resource permit and cost of a land easement.

## DESIGN AND OPERATING ASSUMPTIONS

Three currently or formerly operating wet-storage and depuration facilities were visited and several publications were reviewed for insight in design and operation of real and hypothetical depuration facilities (Bond and Truax 1980, Furfari 1966, Furfari 1991, Howell and Howell 1989, Nielson et al. 1978, Rhodes and Kaswek 1991, Roberts et al. 1991, Williams et al. 1980). This analysis treats the hypothetical depuration facility as an addition to an existing or new shellfish processing operation which purchases the shellfish to be depurated. A separate building is constructed to house the depuration equipment and cooler, but an office and bathroom facilities are located in the existing shellfish operation.

Much of the coastal land in northwest Florida is either state or federally owned, marshy and unsuitable for the facility due to construction and regulatory costs, or does not have road access or access to utilities. The most likely locations are shoreside, adjacent to prohibited waters, within the city limits, and have road, utility, and city sewer access. Process water is derived from a saltwater well because water from areas classified as "prohibited" cannot be used as a source for the depuration facility.

The processing capacity for the plant is a function of the demand for the depurated product, the supply of product, and the number of 48-hour tank cycles that can be made per year. It is not known if there would be a greater demand for depurated product than non-depurated. Estimates for possible plant sizes are based on estimated size of the resource, size of shellfish processors operating in the study area, and length of the operating year. Product source for oysters is CR and RE waters in the study area outside the Suwanee Sound. The baseline operating year is 9 months, with 12 weeks of this time period non-operating due to 10 weeks closure of waters and two weeks down due to maintenance and other reasons. Each tank is used for 2.5 cycles per week. This accommodates the 48-hour depuration period, 6-hour pre-depuration period, and time needed post-depuration for unloading and cleaning tanks. With 2.5 cycles per week, the maximum number of cycles per tank per 9 month operating year is 67.5 (2.5 cycles/tank/week \* 27 weeks). Assuming 5,800 bushels harvested from CR and RE areas, a facility of 214 bushels/week could be supported (5,800 annually/27 weeks). With the addition of the 7,800 bushels from the Suwanee Sound, the weekly capacity increases to 368 bushels/week. Facilities of slightly larger and smaller capacity are also

considered. Product is delivered to the facility by oyster harvesters.

Effluent process water is discharged by pipe directly to the sea. Fresh water used to clean tanks is discharged to the sewer system in accordance with Florida regulations. A mortality of 6% and a count of 280 oysters per 60 pound bushel is used. Laboratory analysis is performed by a state-certified lab operated on-site by the facility.

Estimates of capital and operating costs were made for 12 different capacity facilities (Table 1). While resource assessment surveys indicate the approximate size of facilities that could be supported, different capacities were considered because of the unpredictability of the number of oysters harvested in any season. Environmental conditions, profitability of alternative enterprises, and size of harvests in other areas are 3 factors affecting the supply of oysters for depuration.

Each depuration unit has its own water treatment system (irradiation and filter units) but greater than one tank can be used with each unit. The twelve facility designs are based on one to four depuration units per facility and three different tank sizes. Annual production capacity ranges from 810 to 12,960 bushels. All options keep process waters distinct between depuration units and batches of depurated shellfish. Options 1-3, 5-7, and 9-11 consider one to three depuration units with one tank each. Options 4, 8, and 12 have two units with two tanks per unit sharing process water.

## EXPLANATION OF COSTS CALCULATIONS

The cost budget for Option 6 is provided in Table 2. Option 6 is used for reference purposes only and was not chosen because it was the "best" of all options. Costs for each option are given in Table 3. An example facility layout for design option 6 is given in Figure 1.

*Fixed costs* are incurred regardless of whether depuration occurs. Fixed costs are given in three forms: (1) an initial investment cost (2) a total annual cost (3) a cost per depuration cycle. One depuration cycle is equal to full capacity use of the facility for one 48-hour depuration period.

Building size is based on the area needed for tanks and workspace. The building is screened and has a metal roof. The walls to a height of three feet above the floor are of fiberglass coated

TABLE 1.

Depuration facility design options and associated tank number, size, production capacity, and cycles per operating years.

Design Options	Number of Tanks	Tank Size	Per Tank Capacity (bushels)	System Capacity per Cycle (bushels)	Weekly Production (bushels)	Cycles per Year	Annual Capacity (bushels)
1	1	4.5' * 8' * 3.5'	12	12	30	67.5	810
2	2	4.5' * 8' * 3.5'	12	24	60	135	1620
3	3	4.5' * 8' * 3.5'	12	36	90	202.5	2430
4	4	4.5' * 8' * 3.5'	12	48	120	270	3240
5	1	4.5' * 16' * 3.5'	24	24	60	67.5	1620
6	2	4.5' * 16' * 3.5'	24	48	120	135	3240
7	3	4.5' * 16' * 3.5'	24	72	180	202.5	4860
8	4	4.5' * 16' * 3.5'	24	96	240	270	6480
9	1	6' * 24' * 3.5'	48	48	120	67.5	3240
10	2	6' * 24' * 3.5'	48	96	240	135	6480
11	3	6' * 24' * 3.5'	48	144	360	202.5	9720
12	4	6' * 24' * 3.5'	48	192	480	270	12960

**TABLE 2.**  
Cost budget for depuration facility, Option 6, one depuration cycle.

	Investment	Cost/Year	Cost/Cycle	% of Total
<b>Fixed Costs</b>				
building	\$12,600	\$1,337	\$9.90	2.51%
property	\$18,000	\$1,909	\$14.14	3.59%
equipment	\$12,945	\$1,249	\$9.25	2.35%
materials	\$5,686	\$1,588	\$11.76	2.98%
lab analysis	\$27,250	\$4,651	\$34.00	8.74%
water supply	\$3,468	\$163	\$5.12	0.31%
other	\$5,557	\$2,885	\$21.37	5.42%
<b>SUBTOTAL</b>	<b>\$85,506</b>	<b>\$13,782</b>	<b>\$105.54</b>	<b>25.89%</b>
<b>Variable Costs</b>				
labor		\$23,390	\$173.26	43.94%
utilities		\$4,782	\$35.42	8.98%
maintenance		\$280	\$1.00	0.53%
lab materials		\$9,450	\$70.00	17.75%
water supply		\$733	\$3.20	1.38%
misc.		\$810	\$6.00	1.52%
<b>SUBTOTAL</b>		<b>\$39,445</b>	<b>\$288.88</b>	<b>74.11%</b>
<b>TOTAL COSTS</b>		<b>\$53,227</b>	<b>\$394.42</b>	
<b>COST PER BUSHEL</b>		<b>\$17.44</b>		
<b>COST PER OYSTER</b>		<b>\$0.06</b>		

plywood, which facilitates cleaning. The cost of \$28 per square foot includes the concrete slab, electrical wiring and plumbing, and fluorescent lighting.

Land cost is based on the average cost per square foot for waterfront land and is estimated at \$20 per square foot. Number of square feet of property purchased is equal to double the square footage of the building. Building and property are amortized over a 30 year period at 10% interest.

Depuration tanks are made of 2-inch insulated fiberglass. Tanks are intentionally oversized by 20% to allow for makeup water. Tank sizes and water and shellfish volumes are given in Table 1.

Pumps, sand filter, and UV were chosen on the basis of the required gallons per minute flow. A separate filter and UV are required for each depuration unit. Pumps, sand filters, and UV

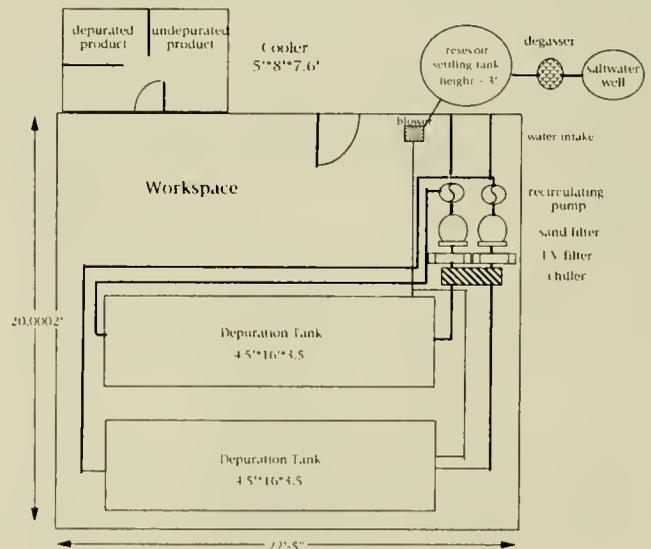
units for facilities with greater than one unit are simply replicates of these items for one unit. The combined pump, filter, and UV cost for Option 2 is, therefore, exactly double that of Option 1. This is an inefficient use of equipment, but is required if unit waters are to be kept separate. Options other than Options 4, 8, and 12 have one tank per unit; Options 4, 8, and 12 have two tanks per unit.

The chiller is the most costly component of the depuration equipment (other than the walk-in cooler). A custom-made chiller minimizes cost while permitting isolation of the process water in each unit.

The walk-in cooler for storage of product has a minimum size of 5' x 8' for the smallest system, increasing to a maximum size

**TABLE 3.**  
Summary of depuration costs per 60 lb bushel by design option assuming 6% mortality.

Design Option	Capacity/Week	Fixed Costs	Variable Costs	Total Costs
1	30	\$12.87	\$29.94	\$42.81
2	60	\$7.65	\$21.34	\$28.98
3	90	\$6.17	\$19.89	\$26.05
4	120	\$5.29	\$13.19	\$18.48
5	60	\$7.34	\$16.85	\$24.18
6	120	\$5.59	\$12.85	\$17.44
7	180	\$3.93	\$12.45	\$16.38
8	240	\$3.46	\$8.75	\$12.20
9	120	\$4.59	\$10.33	\$14.92
10	240	\$3.28	\$8.70	\$11.97
11	360	\$3.00	\$8.83	\$11.83
12	480	\$2.68	\$6.57	\$9.25



**Figure 1.** Depuration facility layout, design option 6.

of 12' \* 16' for the largest. The cooler has dividers to separate depurated from undepurated product.

Materials include costs for PVC components, tank trays, air diffusers, UV bulb replacement, and washing/culling tables. Size and number depends on system capacity.

Lab analysis on samples of process water and meat is required for each cycle for each depuration unit. In comparison to an outside laboratory charge of \$275 per cycle per unit, it is more cost effective for the facility to do its own lab analysis. The limit to its ability to do the analysis is largely dependent on whether available technical expertise in the form of a trained microbiologist is available.

Other fixed costs include property and liability insurance, and property taxes based on local costs. Costs of certification and permitting include costs for shellfish lost during the pre-startup verification, and costs for permitting and easements.

*Variable costs* are the cash expenses directly related to production. Variable costs are calculated per depuration cycle and annually. Labor costs increase at a decreasing rate using a part-time to full-time manager, assistant, and hourly labor of a helper and lab technician. Charges for utilities, building maintenance, and cleaning and miscellaneous supplies are based on system size. Variable costs of laboratory analysis are based on a charge per depuration batch.

## RESULTS AND DISCUSSION

Total investment costs range from \$60,308 to \$203,958 for capacities ranging from 30 bushels per week to 498 bushels per week. Capital and operating costs per 60 pound, 280 count bushel range from \$42.81 for the smallest capacity facility to \$9.25 for the largest, resulting in a cost of \$0.153 to \$0.033 per oyster, respectively. An increase in capacity lowers costs per unit by spreading out fixed costs over a greater number of units. Fixed costs do not increase at a rate equal to that of the increase in the production capacity, and thus the cost per unit decreases. However, fixed costs constitute less than one-third of total costs for each of the depuration design options. The decrease in costs per

unit as system size increases is also attributable to more efficient use of labor and the spreading out of laboratory materials costs over a greater number of bushels when units of greater capacity are used. Thus, economies of scale exist. The combined cost of labor and lab analysis is greater than 50% of total costs for all design options.

While systems with larger capacities entail significantly more labor hours to handle oysters before and after the depuration period, the assistant's, manager's, and lab technician's labor are used more efficiently and thus costs increase at a rate less than the increase in capacity. Labor time used to monitor the filling of tanks or to periodically monitor dissolved oxygen or turbidity does not increase significantly based on larger tank sizes and number of tanks. Owner/manager and lab technician labor also increases at a rate less than the increase in capacity.

The cost for lab materials used for the analysis of each sample is a multiple of the number of depuration cycles. The variable cost per depuration unit per cycle is divided by the number of bushels per unit to calculate the cost per bushel, and thus this cost per bushel decreases as the capacity of each unit increases.

Assuming the same quantity of incoming raw product, lab costs and production scheduling make systems with larger tanks or smaller tanks operating as a single unit more cost efficient than the use of more numerous units. Because of the scheduling of 48-hour depuration periods, systems with greater than two depuration units do not provide any significant degree of flexibility, while costs for equipment and laboratory analysis increase. Based on the 48-hour depuration period and time required for pre- and post-depuration procedures, maximum usage of tanks and equipment is 2.5 cycles per week per tank. If the facility has greater than two depuration units, the operator must load greater than one unit per day to achieve maximum use of equipment. By using larger units (larger tanks or more tanks per unit) and equipment with greater gallons per minute pumping capacity, economies of scale are achieved and lab analysis cost per bushel decreases. For example, Options 6 and 9 have the same 120 bushel capacity per week, but the cost per bushel of Option 6, which has two units of one tank each, is 17%

TABLE 4.

Sensitivity of the cost of depuration, per bushel, to changes in tank utilization and mortality.

Design Options	Tank Utilization						
	100%	94%	90%	80%	70%	60%	50%
1	\$40.39	\$42.81	\$44.43	\$48.47	\$52.50	\$56.54	\$60.58
2	\$27.34	\$28.98	\$30.08	\$32.10	\$35.54	\$38.28	\$41.01
3	\$24.57	\$26.05	\$27.03	\$29.49	\$31.94	\$34.40	\$36.86
4	\$17.43	\$18.48	\$19.18	\$20.92	\$22.67	\$24.41	\$26.15
5	\$22.81	\$24.18	\$25.09	\$27.37	\$29.65	\$31.93	\$34.22
6	\$16.45	\$17.43	\$18.09	\$19.74	\$21.38	\$23.02	\$24.67
7	\$15.45	\$16.38	\$16.99	\$18.54	\$20.08	\$21.63	\$23.17
8	\$11.51	\$12.20	\$12.66	\$13.81	\$14.96	\$16.11	\$17.27
9	\$14.08	\$14.93	\$15.49	\$16.90	\$18.31	\$19.71	\$21.12
10	\$11.30	\$11.97	\$12.42	\$13.55	\$14.12	\$15.81	\$16.94
11	\$11.16	\$11.83	\$12.47	\$13.39	\$14.51	\$15.62	\$16.74
12	\$8.73	\$9.25	\$9.60	\$10.47	\$11.35	\$12.22	\$13.09
	0%	6%	10%	20%	30%	40%	50%

corresponding mortality

TABLE 5.

Sensitivity of the cost of depuration to a change in the number of cycles per year.

Design Options	Weeks Operating					
	45	37	27	23	19	15
1	\$32.28	\$35.70	\$42.81	\$47.39	\$53.89	\$63.87
2	\$23.03	\$24.96	\$28.98	\$31.57	\$35.25	\$40.88
3	\$21.26	\$22.81	\$26.05	\$28.13	\$31.09	\$35.63
4	\$14.67	\$15.90	\$18.48	\$20.14	\$22.50	\$26.11
5	\$18.32	\$20.22	\$24.18	\$26.73	\$30.35	\$35.90
6	\$13.93	\$15.07	\$17.43	\$18.96	\$21.12	\$24.44
7	\$13.44	\$14.39	\$16.38	\$17.65	\$19.47	\$22.25
8	\$9.75	\$10.55	\$12.20	\$13.27	\$14.78	\$17.10
9	\$11.39	\$12.54	\$14.93	\$16.46	\$18.65	\$21.99
10	\$9.62	\$10.38	\$11.97	\$13.00	\$14.45	\$16.68
11	\$9.75	\$10.43	\$11.83	\$12.73	\$14.01	\$15.97
12	\$7.43	\$8.02	\$9.25	\$10.04	\$11.17	\$12.89

greater than the cost per bushel for Option 9, which has one unit of one tank.

The disadvantage of using units with greater capacity is the possibility that the number of bushels available for depuration is less than anticipated. This will result in an increase in the costs per bushel. If the decrease in number of bushels is manifested in a fewer number of bushels per unit, fixed as well as variable costs associated with each cycle are spread out over a fewer number of bushels, significantly increasing the costs per bushel. This is the same type of cost increase per bushel that would occur if significant mortalities were experienced. If the decrease in number of bushels manifests itself in a fewer number of cycles, but with units loaded to capacity on those cycles, the increase in costs per bushel is somewhat less; variable costs per cycle, like some labor and lab materials costs, are not incurred.

Sensitivity analysis was performed to examine the sensitivity of total cost to a change in a particular parameter, with the value of all other parameters remaining constant. Table 4 illustrates the change in costs due to a change in tank utilization which may result from (1) insufficient product initially stocked in tanks or (2) higher tank mortalities during the depuration cycle. To minimize costs under conditions where supply is less than expected, it is preferable for the facility to arrange for delivery of product so that tanks can be filled to capacity.

Table 5 gives the change in costs per bushel when a different number of cycles per year is used. The change in number of cycles is represented by the change in number of weeks operating per year. A greater number of operating weeks per year raises the annual operating capacity. A longer operating year implies a source of product from aquaculture or non-local sources. Forty-five weeks may be a reasonable operating year if the facility has

product available from various sources. Thirty-seven weeks is the operating year estimated for a facility which also receives product from the Suwanee Sound. For Option 6, the cost per bushel falls by 11% when the operating year is lengthened from 27 to 37 weeks. With 37 operating weeks per year, capacity ranges from 1,110 bushels per year for Option 1 to 17,760 bushels for Option 12.

Fewer weeks operating per year could result from a smaller than expected supply of shellfish due to more attractive alternative enterprises for oyster harvesters, or closure of harvest waters due to environmental conditions. As discussed above, a shorter operating year has less of an impact on costs per bushel than a change in mortality.

#### SUMMARY

The economic feasibility of any of these hypothetical depuration facilities depends on the recovery of the cost of depuration by either a premium on sales or a savings on the raw product used for depuration. A large, steady supply of shellstock to the depuration facility can reduce the costs of depuration, and thus a smaller premium (or margin) is required to make the facility self-sustaining or profitable. For the area of study, a collection of fishing communities in northwest Florida that supply approximately 15% of the state's oysters, oyster depuration is not an economically feasible venture because it lacks the means to pay for the cost of depuration and the large shellstock supply to lower costs per unit.

As discussed above, at current prices there exists approximately \$1.80/bu that could realistically be assumed to cover the costs of depuration. However, none of the options yields a cost per bushel less than \$9.25. If oyster prices rose significantly, depuration could become an attractive means to obtain additional resource. High hard clam (*Mercenaria mercenaria*) prices in the late 1980s, accompanied by abundant resource in restricted waters, led to construction of several facilities on the east coast of Florida which depurated clams from these waters. During this period the cost of clam depuration was recovered by purchasing clams from restricted waters at a lower price (Adams 1958). In other regions of the U.S. where the difference between shellstock price for oysters from restricted and approved waters is great, there may be a sufficient margin to cover the costs of depuration.

If depurated product is more attractive to consumers based on a more appealing appearance or perception of enhanced quality or safety, a price premium on depurated product may cover the costs of depuration. Based on available marketing information, however, it is still unclear whether a market or premium price for depurated product exists.

The costs per bushel will decrease as system size increases. It may be possible to reduce costs to less than the \$9.25 per bushel if systems of 1,000 or more bushels per week are used. However, the total size of the resource in the area of study is not sufficient to support a facility larger than those considered here.

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## MODELLING DISEASED OYSTER POPULATIONS I. MODELLING *PERKINSUS MARINUS* INFECTIONS IN OYSTERS

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**ABSTRACT** The endoparasite, *Perkinsus marinus*, is one of the primary factors affecting the abundance and productivity of populations of the Eastern oyster, *Crassostrea virginica*. The environmental and biological factors that control the prevalence and infection intensity of *P. marinus* were examined with a coupled oyster population-parasite model. The simulations show that food availability to the host and temperature control on the growth and developmental rates of the host and parasite are the environmental factors that regulate the parasite. In addition, a feedback between the density of the parasite in the host and the parasite's division rate partially controls infection intensity.

Simulations that use summer temperatures typical of temperate estuaries show increased parasite growth rate which results in more of the net production of the oyster population going to reproduction, thereby reducing the ability of the oyster to outgrow the parasite. As a result, disease prevalence and infection intensity are maximal during the summer throughout the range of environments tested with the model. The simulations suggest that oyster mortality from *P. marinus*, as a fraction of the adult population, may be most pronounced for mid-latitude systems. The increased reproductive potential of oyster populations living at lower latitudes balances increased mortality from disease. Thus, disease prevalence and infection intensity average higher in the simulated populations that correspond to the central part of the latitudinal range of the oyster.

The timing and magnitude of food supply for the oyster greatly affects the degree of mortality produced by the parasite in the simulated populations. In the early spring, the oyster has not switched to a reproductive mode, and more of the net production goes to somatic tissue development. Hence, if increased food is available in the early spring (a March to April spring bloom), mortality due to the parasite decreases in the simulated oyster population. The simulations suggest that the primary defense for the oyster is to simply outgrow *P. marinus*.

The simulated oyster populations remain at moderate to moderately heavy infections for extended periods during the summer for two reasons. First, increasing density of the parasite results in a decreased rate of parasite division, which decreases the rate of oyster host mortality. Second, the rate at which infected individuals reach a lethal infection level is slowed enough that less heavily-infected oysters grow up to replace those that die. These simulation results show that, unlike many host-parasite systems, high infection rates in oysters do not necessarily result in high host mortality. The simulations further suggest that the rates of *P. marinus* division and mortality must vary regionally among oyster populations and that some lability in disease resistance by the oysters must be present.

The results from the oyster population-*P. marinus* model imply that environmental changes, such as temperature variability, which may result from climatic effects, can potentially alter the prevalence and infection levels of the parasite in oyster populations. Sensitivity of the host-parasite system to the recent history of environmental change provides a mechanism for exaggerating the effects of extreme years, such as times of reduced food supply or extended periods of warm temperature. Thus, any management strategy for diseased oyster populations must take into account environmental conditions, the physiology of the oyster and the parasite, as well as the previous history of the disease in the population.

**KEY WORDS:** Oyster, *Crassostrea virginica*, dermo, *Perkinsus marinus*, mathematical model, host-parasite interactions

### INTRODUCTION

Disease is one of the factors that can regulate the abundance and productivity of animal populations (Gill 1928, Ackerman et al. 1984, Kermack and McKendrick 1991a, Kermack and McKendrick 1991b). Much research has gone into the study of disease effects on human and many terrestrial animal populations. Until recently, the regulating effect of disease in natural marine populations has not been well documented or studied. It is now recognized that the endoparasite protozoan, *Perkinsus marinus*, is one of the primary factors affecting populations of the Eastern oyster (*Crassostrea virginica*) (Andrews 1988, Andrews and

Ray 1988). Yearly mortality rates due to *P. marinus* normally exceed 50% of the adult oyster population. Epizootics that produce mass mortalities in oyster populations are well known throughout most of the latitudinal range of *P. marinus* (Powell et al. submitted a) and are a potentially important factor determining ecological change in estuaries and economic performance of the oyster shellfishery (e.g., Bosch and Shabman 1990, Mann et al. 1991, Lipton et al. 1992).

Transmission rates of *P. marinus* are very high. Nearly 100% of new recruits acquire the disease within 6 months or less in most Gulf of Mexico estuaries (Craig et al. 1989, Wilson et al. 1990). Disease infection intensity in oyster populations follows a well defined seasonal cycle. The disease intensifies in the spring and summer, peaks in mid to late summer, and declines in the fall and winter. Most mortality due to the disease occurs in the late summer

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in the adult portion of the oyster population. *P. marinus*-induced mortality in juvenile size classes is rare.

The deleterious effects of infection by *P. marinus* can be alleviated somewhat by ambient environmental conditions, particularly variations in temperature and salinity (Mackin 1955, Soniat 1985, Soniat and Brody 1988). Low temperatures and salinities reduce prevalence and infection intensity. For example, the normal salinity gradient encountered in most estuarine systems produces gradients in disease severity during the summer. Warmer winter water temperatures result in increased disease incidence in the following year. A recent study (Powell et al. 1992) suggested that yearly changes in oyster population health in the Gulf of Mexico are linked to climatic cycles, such as those associated with El Niño-Southern Oscillation events, which control rainfall and, consequently, salinity. However, bay-to-bay variation in disease prevalence and infection intensity that occurs in oyster populations in the Gulf of Mexico is relatively unrelated to salinity fluctuations (Wilson et al. 1990, Powell et al. 1992). Mass mortalities can occur in some bays at salinities that do not significantly affect oyster populations elsewhere (Powell et al. submitted b). Therefore, interactions between environmental conditions, disease, and oyster population dynamics are complex.

This study presents a coupled host-parasite model that was developed to investigate environmental and biological factors that affect the prevalence and infection intensity of *P. marinus* in oyster populations. A series of simulations are presented that are designed to investigate the effects of variations in temperature, salinity, oyster food supply, and turbidity level on this disease in oyster populations. Additional simulations consider the role of *P. marinus* density and mortality in determining the level of disease infection. The final set of simulations are directed towards understanding recruitment and disease transmission rates and system memory effects on disease intensity. Most of the simulations use environmental conditions from Galveston Bay, Texas, a temperate mid-latitude bay where *P. marinus* infects almost 100% of the oyster population. Variations in the Galveston Bay conditions are introduced by inclusion of environmental time series from other estuarine systems. The host-parasite model is described in detail in the following section. The results and discussion of the simulations are then presented. The final section gives the conclusions from this modeling study.

## THE OYSTER POPULATION-PERKINSUS MARINUS MODEL

### General Characteristics

The host-parasite model (Fig. 1) consists of separate models for the dynamics of the postsettlement oyster population and the growth of *P. marinus*. The two models are coupled by relationships that describe the removal of oyster energy by the parasite to support its metabolic requirements and relationships that relate the rates of parasite division and mortality to host mortality. The oyster population model, described in detail by Hofmann et al. (1992, 1994) and Powell et al. (1992, 1994, in press), consists of a size-structured model that considers the processes regulating the growth and death of the oyster from newly settled juveniles to adults. The description of this model will focus on only the modifications made to allow connection between the parasite and host components. The *P. marinus* model includes metabolic growth and loss processes as well as a component that describes the transmission of the disease. The parasite model, which uses infection

level as the state variable, is described in detail following a brief review of the oyster population model.

### Governing Equation

The time change in oyster standing stock ( $O_{j,k}$ ) in each oyster size class ( $j$ ) and *P. marinus* infection level ( $k$ ) is the result of changes in net production ( $NP_{j,k}$ ), which is the sum of the production of somatic ( $Pg_{j,k}$ ) and reproductive ( $Pr_{j,k}$ ) tissue, and the addition of individuals from the previous size class or loss to the next largest size class by growth. Oyster net production is assumed to be the difference between assimilation ( $A_{j,k}$ ) and respiration ( $R_{j,k}$ ), as discussed by White et al. (1988), and losses to *P. marinus* ( $E_{j,k}$ ) as:

$$NP_{j,k} = Pg_{j,k} + Pr_{j,k} = A_{j,k} - R_{j,k} - E_{j,k}. \quad (1)$$

The governing equation for the oyster population is then:

$$\frac{dO_{j,k}}{dt} = Pg_{j,k} + Pr_{j,k} + (\text{gain from } j-1) - (\text{loss to } j+1) \quad (2)$$

where  $j = 1, 11$ , which represents the size class partitioning of the oyster life history (Table 1). Reproductive tissue formation is 0 for the first 3 size classes, which represent juveniles.

During suboptimal conditions, oysters can resorb gonadal or somatic tissue and hence lose biomass ( $NP_{j,k} < 0$ ) and transfer into the next lower size class. Thus biomass can change during periods of negative scope for growth, which is the basis for the use of condition index as a measure of health in oysters (e.g., Newell 1985, Wright and Hetzel 1985). To allow for this, equation (2) is modified as:

$$\begin{aligned} \frac{dO_{j,k}}{dt} = & Pg_{j,k} + Pr_{j,k} + (\text{gain from } j-1) - (\text{loss to } j+1) \\ & + (\text{gain from } j+1) - (\text{loss to } j-1). \end{aligned} \quad (3)$$

The last 2 terms on the right side of equation (3) represent the individuals losing biomass and, thus, moving to the next lower size class.

The final modification to the oyster governing equation allows oysters in any size class to increase or decrease in *P. marinus* infection intensity:

$$\begin{aligned} \frac{dO_{j,k}}{dt} = & Pg_{j,k} + Pr_{j,k} + (\text{gain from } j-1) - (\text{loss to } j+1) \\ & + (\text{gain from } j+1) - (\text{loss to } j-1) \\ & + (\text{gain from } k-1) - (\text{loss to } k+1) \\ & + (\text{gain from } k+1) - (\text{loss to } k-1). \end{aligned} \quad (4)$$

The last 4 terms in equation (4) represent changes in infection intensity of the oyster population as the *P. marinus* population increases or decreases in number. The model includes 28 predefined infection levels. Level 1 consists of uninfected oysters. The remaining 27 levels represent degrees of infection that correspond to the number of doublings of the *P. marinus* population beginning with one cell in level 2.

Three aspects of the model given by equation (4) deserve note. First, settlement of juvenile oysters (as spat) occurs exclusively in the first size class and first infection level. These newly recruited individuals are uninfected by *P. marinus*. Second, movement of oysters from the uninfected to the newly infected stage occurs by the acquisition of one infective cell (infection level 2) and occurs only in the positive direction, a gain of infection. Infections, once

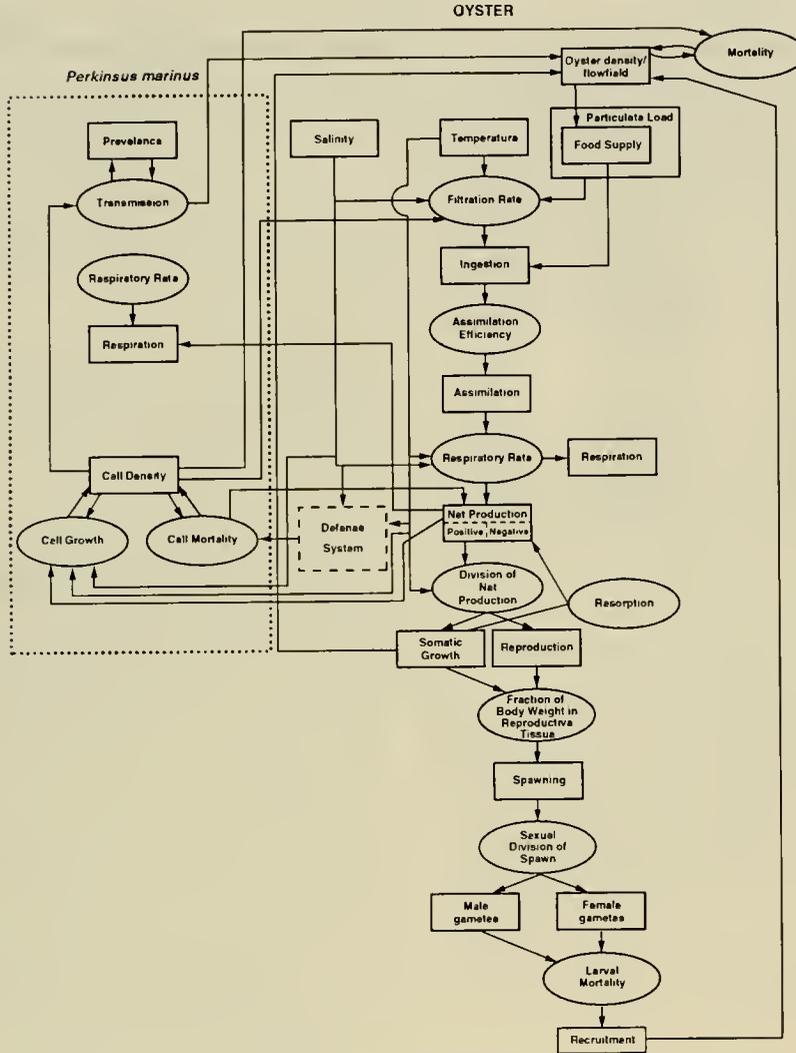


Figure 1. Schematic of the coupled oyster population-*Perkinsus marinus* model. See text for a detailed description of the model components.

acquired by oysters, are never lost (Andrews 1988, Ragone Calvo and Burreson 1994). Finally, once the oysters have reached the infection level defined as lethal (Table 1), they are classified as dead and the *P. marinus* and the oyster biomass are permanently removed.

The gain, loss or transfer of energy (or biomass) between size classes or across infection levels in equation (4) is expressed in terms of specific rates ( $d^{-1}$ ) which are multiplied by the caloric quantity in the size or infection class. Transfers of oysters between size classes were scaled by the ratio of the average weight of the current size class (in *g dry wt* or *cal*) to that of the size class from which energy was gained or to which energy was lost. This scaling is necessary because the oyster size classes were unevenly distributed across the size-frequency spectrum. This ensured that the total number of oyster individuals in the model was conserved, in the absence of recruitment and mortality. A similar scaling is used for transfers between infection levels because these are not equivalent in dimension. Thus, each specific rate for each transfer was scaled by:

$$\text{for transfers up: } W_j / (W_{j+1} - W_j)$$

$$\text{for transfers down: } W_j / (W_j - W_{j-1})$$

where  $W$  is the median value for biomass (*gAFDW*) in the size class, or by the ratio of parasite number between infection classes:

$$\text{for transfers up: } C_k / (C_{k+1} - C_k)$$

$$\text{for transfers down: } C_k / (C_k - C_{k-1})$$

where  $C$  is the number of cells of *P. marinus* per oyster individual. For simplicity, these scalings are not explicitly stated in the equations given in the following sections.

**The Oyster Model**

The model includes parameterizations for the processes that determine the production of somatic and reproductive tissue and thus the transfer between size classes. Specifically included are formulations for: assimilated ingestion as it depends on filtration rate, ambient food supply and assimilation efficiency; filtration rate as a function of oyster size, temperature, salinity, turbidity and current flow; respiration as it depends upon oyster size, temperature and salinity; the apportionment of net production into somatic and reproductive growth as a function of temperature and time of year; the preferential resorption of gonadal tissue when  $NP_{j,k} < 0$ ; and spawning as a function of the total cumulative

TABLE 1.

Biomass and length dimensions and lethal *Perkinsus marinus* parasite density of the oyster size classes used in the model.

Model Size Class	Biomass (g ash free dry wt)	Length (mm)	Lethal Parasite Density (cells oyster <sup>-1</sup> )
1	1.3 × 10 <sup>-7</sup> -0.028	0.3-25.0	2 <sup>21</sup>
2	0.028-0.10	25.0-35.0	2 <sup>23</sup>
3	0.10-0.39	35.0-50.0	2 <sup>24</sup>
4	0.39-0.98	50.0-63.5	2 <sup>25</sup>
5	0.98-1.44	63.5-70.0	2 <sup>26</sup>
6	1.44-1.94	70.0-76.0	2 <sup>26</sup>
7	1.94-3.53	76.0-88.9	2 <sup>26</sup>
8	3.53-5.52	88.9-100.0	2 <sup>27</sup>
9	5.52-7.95	100.0-110.0	2 <sup>27</sup>
10	7.95-12.93	110.0-125.0	2 <sup>27</sup>
11	12.93-25.91	125.0-150.0	2 <sup>27</sup>

Biomass is converted to size using the relationship given in White et al. (1988).

reproductive biomass and the male/female ratio. The relationships used for these processes are shown in Table 2.

The modifications made to the oyster population model so that it could be interfaced with the *P. marinus* model consisted of including sources of natural mortality so that the effect of these processes could be assessed relative to the mortality produced by *P. marinus*. Larval mortality, mortality due to predation, and mortality due to low salinity were identified as the primary sources of natural mortality and were added to the model as follows.

While in the plankton, oyster larvae undergo considerable mortality from a variety of sources, which reduces the number of individuals that are recruited to the postsettlement population from a spawn. Oyster larval mortality was included using a linear relationship of the form:

$$\text{number of larvae recruited spawn}^{-1} = s (\text{eggs spawned}) \quad (5)$$

where  $s$  determines the rate at which individuals are lost per spawn. No attempt was made to differentiate among the many sources of planktonic mortality.

Natural mortality of the post-settlement population was also specified with a linear relationship of the form:

$$M(p) = k_p (\text{number of living oysters}) \quad (6)$$

where  $M(p)$  is the number of individuals that die in a given time interval and  $k_p$  is the daily mortality rate (d<sup>-1</sup>). As with larval mortality, this approach does not differentiate among the many sources of oyster mortality. For the simulations presented here, equation (6) was used to produce mortality for the juvenile oyster size classes to complement the adult mortality produced by *P. marinus*.

Low salinity is a principle cause of catastrophic mortality of postsettlement oyster populations during some flood years (Hofstetter 1977, Ray 1987, Soniat and Brody 1988). Wells (1961) and Chanley (1957) provide survivorship data at low salinity for temperatures greater than 20°C, which show that salinities lower than 6 ppt produce mortality at summer temperatures and that the rate of mortality rises as salinity declines below 6 ppt. Additionally, observations given in Gunter (1955) and those from Galveston Bay (Powell, unpublished data) show that oyster survivorship increases

substantially at low salinity as temperature declines. Therefore, the temperature-dependent mortality produced by salinities lower than 6 ppt was modeled as:

$$M(s) = k_s (\text{number of living oysters}) \quad (7)$$

where  $M(s)$  is the number dying per time and  $k_s$  in d<sup>-1</sup> is given by

$$k_s = (\alpha_1 S + \beta_1)T + (\alpha_2 S + \beta_2). \quad (8)$$

Salinity,  $S$ , is given in ppt and temperature,  $T$ , is in °C. Coefficient values are given in Table 2.

#### The *Perkinsus marinus* Model

The *P. marinus* model includes processes that govern parasite growth and mortality, those that determine the energy demand of the parasite on the host, and those that affect the physiology of the host. The relationships used to describe these processes are given in the sections that follow.

#### *Perkinsus marinus* Growth and Mortality

Cell division time is the time between one cell division and the next for an individual cell. The population doubling time, however, depends upon the balance between the rate of cell division and the rate of cell mortality. *P. marinus* mortality is likely mediated in some way by the defense system of the oyster (Saunders et al. 1993). In the *P. marinus* population model, the biology of the parasite and the processes determining its rate of division are treated separately from those that describe the oyster's defense system and the rate of parasite mortality.

Measurements of *P. marinus* division time are limited and the effects of temperature, salinity and parasite density on this are poorly known. However, information from Ray (1954a) and Mackin and Boswell (1954) suggest that division times for the parasite range from 7 to 60 hours. More recently, Choi et al. (1989) estimated a doubling time of 7 hours. The fastest rate of division, at low parasite density, observed by Saunders et al. (1993) ranged between 4 and 10 hours at 30°C and 17 ppt.

Given the limited observations on *P. marinus* growth *in vivo*, this process was modeled using standard relationships for temperature and salinity dependencies which were calibrated by comparing the simulated growth of *P. marinus* to data sets that provide observations of the time dependency of parasite infection intensity. These data came from April Fools Reef in Galveston Bay, TX (Soniat 1985), Biloxi Bay, MS (Ogle and Flurry 1980), and North Inlet, SC (Crosby and Roberts 1990). The disease model remains to be verified for Mid-Atlantic estuarine systems and it may not be valid where *Haplosporidium nelsoni* is also present.

Temperature control on the specific rate of parasite division,  $r_d(T)$ , was assumed to follow a standard exponential form:

$$r_d(T) = r_{d0} e^{\alpha(T(t) - T_0)}. \quad (9)$$

To calibrate equation (9) a known division rate at a given temperature is needed. Observations of field populations suggest that infection intensity begins to rise in most populations when the temperature exceeds 20°C and the salinity exceeds 20 ppt. Therefore, the 20°C-20 ppt boundary was used to standardize parasite division and mortality rates. At 20°C and 20 ppt, parasite division should just balance loss (Ray 1954a, Mackin 1962, Andrews 1988). The division time at 20°C and 20 ppt was determined to be 30 hours by comparing simulated distributions to those in Soniat (1985), Ogle and Flurry (1980) and Crosby and Roberts (1990). This division time is within the ranges of those reported from the limited laboratory measurements. A  $Q_{10}$  of 2.0, which is consis-

TABLE 2.  
Primary equations used for the postsettlement oyster population model.

Equations	Comments and Parameter Definitions
<p>Filtration rate and water flow</p> $\frac{\partial F}{\partial t} + \frac{\partial(uF)}{\partial x} + \frac{\partial(wF)}{\partial z} - \frac{\partial^2(AF)}{\partial z^2} + \alpha FO = 0$	<p>Flow limitation on food supply is calculated using a volume of water over the bottom with length and width, <math>L</math>, and height, <math>h</math></p> <p><math>F</math>, food  <math>u</math>, horizontal advective velocity  <math>w</math>, vertical advective velocity  <math>A</math>, vertical diffusion coefficient  <math>\alpha</math>, total filtration rate summed over all oyster size classes,</p> $\sum_{j=1}^n FR_{D_j}$ <p><math>O</math>, oyster biomass                      partial derivatives indicate changes in time (<math>t</math>) and in the horizontal (<math>x</math>) and vertical (<math>z</math>) directions</p> <p>Continuity equation</p>
$\frac{\partial u}{\partial x} + \frac{\partial w}{\partial z} = 0$	
<p>Characteristic velocity profile</p> $u(x,z) = u_o(x)\ln(z/z_o)$ $u_o(x) = \hat{u}(x)/\ln(h/z_o)$	<p><math>u_o(x)</math>, a specific horizontal speed at height, <math>z = h</math>  <math>z_o</math>, bottom roughness parameter = 10% of height of oyster clumps  <math>\hat{u}(x)</math>, the specified speed</p>
<p>Food profile</p> $F = F_o(t) + (x/L)F_1(t)$	<p>Food assumed to be independent of height and a linear function of distance across the box. <math>F_o</math>, <math>F_1</math> are food concentrations at the upstream and downstream boundaries of the volume of interest</p>
<p>Food calculation</p> $F_o(t = 0) = F_o(t = \Delta t) = F_{oo}$ $F_1(t = 0) = 0$ $F_1(t = \Delta t) = F_1^1 = [-2\alpha\Delta t F_{oo}O^o + (\Delta t/hL)(D^1 + D^o)] / [1 + 0.5\alpha\Delta t O^o + \{(\hat{u}(L) + \hat{u}(0))(\Delta t/2L)\} [1 - 1/\ln(h/z_o)]]$	<p><math>F</math> is integrated over the volume and the average amount of food in the box during one time step is calculated by differencing over time. <math>F_{oo}</math>, the specified food concentration</p>
<p>Food reduction factor</p> $F_{red} = (F_{oo} + 0.25F_1^1)/F_{oo}$	<p><math>F_{red}</math> is the fraction by which the food concentration is reduced</p>
<p>Food content</p> $f^* = F_{red}F_{oo}$	<p><math>f^*</math> is the available food</p>
<p>Conditions for simulation</p>	<p>Box length, <math>L = 1</math> m                      Thickness of bottom flow, <math>h = 5.4</math> cm</p>
<p>Filtration rate as a function of temperature</p> $FR_j = \frac{L_j^{0.96} T^{0.95}}{2.95}$ $L_j = W_j^{0.117} 10^{0.669}$	<p>filtration rate (<math>FR_j</math>) in ml filtered <math>\text{ind}^{-1} \text{min}^{-1}</math> by a particular oyster size, <math>j</math>; length (<math>L_j</math>) obtained from <math>W_j</math>, the ash-free dry weight in g; <math>T</math>, temperature</p>
<p>Filtration rate as a function of ambient salinity, <math>S</math></p> $FR_{S_j} = FR_j$ $FR_{S_j} = FR_j(S - 3.5)/4.0$ $FR_{S_j} = 0$	<p>at <math>S \geq 7.5</math> ppt                      at <math>3.5 &lt; S &lt; 7.5</math> ppt                      at <math>S \leq 3.5</math> ppt</p>
<p>Filtration rate as a function of turbidity</p> $\tau^* = \tau_{red}\tau_{oo}$ $\tau = (4.17 \times 10^{-4}) (10^{0.0418\tau})$ $FR_{\tau_j} = FR_{S_j} \left[ 1 - 0.1 \left( \frac{\log_{10}\tau + 3.38}{0.0418} \right) \right]$	<p>calculated similarly to <math>f^*</math>.  <math>\tau</math>, total particulate content (inorganic + organic) in <math>\text{g l}^{-1}</math>, <math>x</math>, the percent reduction in filtration rate                      filtration rate with turbidity effects</p>
<p>Ingestion</p> $I_{j,k} = f^* FR_{D_{j,k}}$	<p>ingestion rate (<math>I</math>) as a function of food concentration and filtration rate</p>

continued on next page

TABLE 2.

continued

Equations	Comments and Parameter Definitions
Assimilation $A_{j,k} = I_{j,k}A_{\text{eff}}$	$A_{\text{eff}}$ , assimilation efficiency
Respiration as a function of temperature $R_j = (69.7 + 12.6T)W_j^{b-1}$	respiration rate ( $R_j$ ) for a particular oyster size class in $\mu\text{l } O_2$ consumed $\text{hr}^{-1} (\text{g dry wt})^{-1}$ ; $b = 0.75$
Respiration as a function of salinity $R_r = 0.007T + 2.099$ $R_r = 0.0915T + 1.324$ $R_{T_j} = R_j$ $R_{T_j} = R_j(1 + [15 - S](R_r - 1)/5)$ $R_{T_j} = R_jR_r$	at $T < 20^\circ\text{C}$ at $T \geq 20^\circ\text{C}$ at $S \geq 15\text{ppt}$ at $10\text{ppt} < S < 15\text{ppt}$ at $S \leq 10\text{ppt}$
Reproduction, $R_f$ Juvenile/adult boundary $Pr_{j,k} = R_{\text{eff}j}NP_{j,k}$	0.39 g ash-free dry weight; about 50 mm reproductive tissue development for a given oyster size class as a function of reproductive efficiency, $R_{\text{eff}j}$ , and total net production, $NP_{j,k}$
$R_{\text{eff}j,k} = 0.054T(t) - 0.729$ $R_{\text{eff}j,k} = 0.047T(t) - 0.809$ when $NP_{j,k} < 0$ $R_{fj,k} = 0.20 O_{j,k}$	reproductive efficiency temperature dependence for January to June reproductive efficiency temperature dependence for July to December preferential resorption of gonadal tissue spawning occurs when the reproductive biomass exceeds 20% of total oyster biomass
$f_{\text{ratio}} = 0.021 L_b - 0.62$ number of eggs spawned = $R_{fj,k} \left(\frac{1}{C}\right) \left(\frac{1}{W_{\text{egg}}}\right)$ $W_{\text{egg}} = 2.14 \times 10^{-14} V_{\text{egg}}$	$f_{\text{ratio}}$ , the ratio of females to males; $L_b$ , length in mm number of eggs spawned, $C$ is number of calories per egg, $W_{\text{egg}}$ is egg weight $V_{\text{egg}}$ , oyster egg volume
Larval recruitment	larval planktonic time assumed to be 20 days
Larvae mortality number of larvae recruited $\text{spawn}^{-1} = s$ (number of eggs spawned)	$s$ , the mortality rate, in $\text{spawn}^{-1}$
Post-settlement population natural mortality $M(p) = k_p$ (number of living), for $j = k, l$	$M_p$ , the number dying $\text{time}^{-1}$ $k_p$ , the daily mortality rate ( $d^{-1}$ ); $k$ and $l$ , the inclusive size classes being affected by mortality
Post-settlement salinity mortality $M(s) = K_s$ (number of living) $K_s = (\alpha_1 S + \beta_1 T) + (\alpha_2 S + \beta_2)$	$M_s$ , the number dying $\text{time}^{-1}$ $K_s$ , daily mortality rate ( $d^{-1}$ ) $\alpha_1 = -0.000348$ $\alpha_2 = 0.00232$ $\beta_1 = 0.01764$ $\beta_2 = -0.3089$ $S$ , ambient salinity (ppt) $T$ , ambient temperature ( $^\circ\text{C}$ )
Caloric conversions: oysters food oyster eggs	6100 cal (g dry wt) $^{-1}$ 5168 cal (g dry wt) $^{-1}$ 6133 cal (g dry wt) $^{-1}$

tent with measurements for *P. marinus* (Chu and Greene 1989), is used to calculate a division rate at temperatures other than  $20^\circ\text{C}$ . The coefficients and their values thus determined for equation (9) are defined in Table 3.

The rate of parasite division is independent of salinity except at and below 10 ppt (Chu and Greene 1989, Ragone and Bureson 1993). Thus, for salinities below 10 ppt, equation (9) was modified as:

$$r_d(T, S) = r_d(T_0, S_0) \left(\frac{S}{10}\right) e^{\alpha(T(t)-T_0)} \quad (10)$$

where coefficient definitions and values are given in Table 3. This relationship provides a decrease in parasite division rate at low salinity, but retains the temperature relationship.

Simulations of *P. marinus* using equation (10) resulted in parasite growth rates and densities that were too high relative to those suggested by field measurements in Soniat (1985), Ogle and Flurry (1980), and Crosby and Roberts (1980) under the appropriate environmental constraints. Most measurements of protozoa in culture show that division rate decreases at high population densities as food becomes limiting (Hall 1967). A similar response

TABLE 3.  
Coefficient definitions and values for the *Perkinsus marinus* population model.

Coefficient	Definition	Value	Units
$r_d(T)$	specific rate of parasite division	calculated	$d^{-1}$
$r_{d0}$	base specific parasite division rate	0.555	$d^{-1}$
$\alpha$	$Q_{10}$ conversion	0.06931	$^{\circ}C^{-1}$
$T_0$	base temperature for parasite division rate	20	$^{\circ}C$
$S_0$	base salinity for parasite division rate	20	ppt
$r_{d1}$	base specific parasite division rate	0.555	$d^{-1}$
$\beta$	parasite density scaling factor	$2.454 \times 10^8$	$g\ AFDW\ cell^{-1}$
$C_k$	parasite number	calculated	number of cells
$W_j$	oyster weight	Table 1	$g\ AFDW$
$\gamma$	parasite density scaling factor	-1.5	no units
$r_m(T, S)$	specific parasite loss rate	calculated	$d^{-1}$
$r_{m0}$	base specific parasite loss rate	0.555*	$d^{-1}$
$\delta$	$Q_{10}$ conversion	0.08153	$^{\circ}C^{-1}$
$Ec$	total <i>P. marinus</i> energy demand	calculated	$cal\ d^{-1}$
$Eg$	energy for <i>P. marinus</i> population increase	calculated	$cal\ d^{-1}$
$Er$	energy for <i>P. marinus</i> respiration demand	calculated	$cal\ d^{-1}$
$El$	<i>P. marinus</i> mortality	calculated	$cal\ d^{-1}$
$\epsilon$	conversion	$1.16 \times 10^4$	$hr\ cal\ d^{-1}\ nl^{-1}$
$D$	average parasite cell diameter	8	$\mu m$
$\zeta$	conversion	$9.57 \times 10^{-10}$	$cal\ \mu m^{-3}$
$\omega$	respiration scaling factor	-4.09	$ml\ hr^{-1}\ \mu m^{-3}$
$\theta$	respiration scaling factor	0.75	no units
$\kappa$	filtration scaling factor	0.58	no units
$\lambda$	filtration scaling factor	579	no units
$\mu$	conversion	$-2.287 \times 10^{-4}$	$g\ AFDW\ cell^{-1}$
$FR_{D_j,k}$	filtration rate, infected oyster	calculated	$ml\ oyster^{-1}\ min^{-1}$
$C_{L_j}$	lethal parasite density	calculated	$cells\ oyster^{-1}$
$v$	mortality scaling factor	2.057	no units
$z$	weight scaling factor	$1.3258 \times 10^{-7}$	$g\ AFDW$
$q$	weight scaling factor	0.2625	no units
$\sigma$	mortality scaling factor	3.2	no units
$v$	weight conversion factor	5	$g\ wet\ wt\ (g\ dry\ wt)^{-1}$
$\tau$	infection level scaling factor	1409.9	$cells\ (g\ wet\ wt)^{-1}$
$\phi$	infection level scaling factor	0.64296	no units
$r_t$	specific rate of transmission	calculated	$d^{-1}$
$r_{tb}$	base specific interpopulation transmission rate	0.2	$y^{-1}$
$r_{to}$	base specific intrapopulation transmission rate	12	$y^{-1}$

\* Value modified in some simulations.

by *P. marinus* is suggested by experiments in which the rate of DNA production by *P. marinus* at various cell densities showed lower growth rates at high cell densities (Saunders et al. 1993). Also, a decrease in hemolymph protein in oysters has been noted during summer months when *P. marinus* infection intensity is high (Chintala and Fisher 1991) and as a result of MSX infection (Ford 1986). Using the measurements from Saunders et al. (1993), an empirical relationship that modifies the specific parasite division rate at high cell density,  $r_d(\rho)_{j,k}$ , was derived as

$$r_d(\rho)_{j,k} = \beta r_d(T, S) \left( \frac{C_k}{W_j} \right)^\gamma \quad (11)$$

where  $r_d(T, S)$  is determined from equation (10). Coefficient definitions and values are given in Table 3. In the model, the parasite division rate that is used is the minimum of that determined from equations (10) and (11).

The specific parasite division rate for a range of temperatures and salinities calculated from equation (10) is shown in Figure 2a.

The major decline in parasite doubling time as a result of salinity occurs between 0 and 10 ppt; salinities above 10 ppt have little effect on doubling times at all temperatures. At high temperatures (e.g., 30°C), the salinity effect on parasite doubling time is most pronounced. Below 10°C, which is a typical winter temperature, salinity has little effect on doubling time. As expected, the parasite population doubling time decreases with increasing temperature, from about 60 hours at 20°C to about 30 hours at 30°C.

Parasite mortality was parameterized using measurements made for hemocytes, which are an important component of the oyster's defense mechanism (Fisher 1988). These measurements show that parasite mortality is temperature and salinity dependent (Fisher and Newell 1986, Fisher and Tamplin 1988). Moreover, field (Soniata 1985, Burrell et al. 1984) and laboratory (Fisher 1992) observations show that the effect of salinity on *P. marinus* mortality is discernible only at high temperatures. One explanation for this is that hemocytes are maximally active at low temperature so that salinity changes have little effect. However, at higher temperatures where hemocyte activity is reduced, some capability is

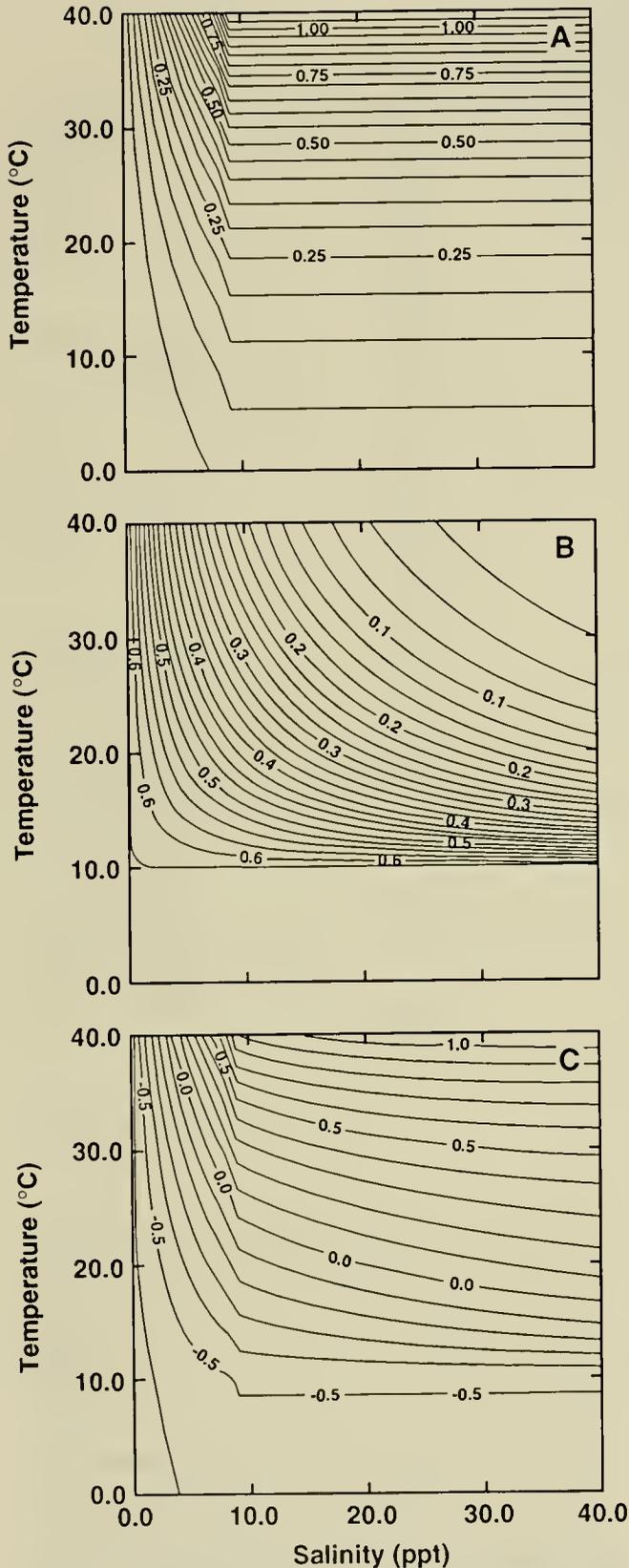


Figure 2. (A) Contours of *Perkinsus marinus* specific growth rate,  $r_d(T, S)$ , for a range of temperatures and salinities. Contour interval is  $0.05 \text{ d}^{-1}$ . (B) Contours of *Perkinsus marinus* specific mortality rate,  $r_m(T, S)$ , for a range of temperatures and salinities. Contour interval is  $0.025 \text{ d}^{-1}$ . (C) Contours of *Perkinsus marinus* population specific growth rate ( $r_d(T, S) - r_m(T, S)$ ). Contour interval is  $0.1 \text{ d}^{-1}$ . All calculations were done assuming a parasite division time of 60 hours at  $20^\circ\text{C}$  and 20 ppt.

recovered when the oyster is exposed to low salinity. Thus, a temperature and salinity dependent relationship for the specific parasite mortality rate,  $r_m(T, S)$ , was obtained using measurements of hemocyte activity reported in Fisher and Newell (1986), Fisher and Tamplin (1988), Fisher et al. (1989, 1992), and Chintala and Fisher (1991) as:

$$r_m(T, S) = r_{m0} e^{-\delta \left( \frac{S(t)}{S_0} \right)^\epsilon} e^{-\delta \left( \frac{T_0 - 10}{S_0} \right) (S(t) - S_0)} \quad (12)$$

where  $\epsilon$  is the larger of the temperature at  $10^\circ\text{C}$  or the difference between the ambient temperature and the base temperature of  $20^\circ\text{C}$  (i.e.,  $\max(10^\circ\text{C}, T(t) - T_0)$ ). The definitions and values for the coefficients in equation (12) are given in Table 3. The value used for  $\delta$  was obtained by applying a  $Q_{10}$  of 2.26 to the base mortality rate. The specific parasite mortality rate assumes no reduction in hemocyte activity at extremely low salinity. Ford and Haskin (1988) found active hemocytes down to 6 ppt. Oyster mortality from low salinity begins at these lower salinities.

Parasite mortality should be dependent on cell density, with the effectiveness of the defense system decreasing at high parasite densities. This is supported by measurements (Anderson et al. 1992b) that showed that the number of hemocytes in heavily infected oysters is only about double that in lightly infected oysters; whereas, the number of *P. marinus* cells is a factor of 1000 or more higher. Thus, the relative activity of the hemocytes must decline at high parasite density. Measurements sufficient to exactly describe the relationship between parasite concentration and parasite mortality are not available. Hence, density effects on mortality rate were assumed to follow the same relationship as was used for the density effect (equation 11) on parasite division rate as:

$$r_m(\rho)_{j,k} = \beta r_m(T, S) \left( \frac{C_k}{W_j} \right)^\gamma \quad (13)$$

Coefficient definitions and values are given in Table 3. The specific parasite mortality rate was taken to be the minimum of the rate calculated using equation (12) and that calculated with equation (13).

The specific parasite mortality rate calculated from equation (12) is shown in Figure 2b. Mortality is high above  $10^\circ\text{C}$  and at low salinity. As temperature and salinity increase, the specific parasite mortality rate decreases, with lowest mortalities occurring at temperatures and salinities that are typical of summer conditions for most mid-latitude estuaries. The formulation for the specific parasite mortality assumes that extremes in temperature and salinity do not directly produce mortality in *P. marinus*. Observations (Goggin et al. 1990) indicate that *P. marinus* is relatively resistant to environmental extremes, and is at least as resistant as the oyster host.

The combined effect of the specific growth and mortality relationships [equations (10) and (12)] on the specific rate of *P. marinus* population growth is shown in Figure 2c. No net population growth (zero contour) occurs at  $20^\circ\text{C}$  and 20 ppt. The population growth rate becomes negative at low temperature regardless of salinity and at low salinity regardless of temperature. Therefore, when subjected to these environmental extremes, the *P. marinus* population decreases in concentration and infection level in an individual oyster decreases. At high salinity and temperature, the specific population growth rate is positive and the population density increases.

#### *Perkinsus marinus* Energy Demand

The *P. marinus* population depends on the oyster host to provide sufficient energy to support its respiration and growth. Thus,

the energy requirement of the parasite population,  $Ec$ , can be expressed as:

$$Ec = Eg + Er - El \quad (14)$$

where  $Eg$  is the energy required to increase the population biomass through cell division and  $Er$  is the energy requirement for population respiration. The last term in equation (14),  $El$ , represents the return of energy to the host from the parasite which occurs through mortality. Although hemocyte exomigration (Cheng 1983) might limit the importance of  $El$ , exomigration was not included in the model. The terms in equation (14) are formulated as described below.

The energy requirement for parasite population growth is defined by  $Eg - El$  and is determined by the net change in parasite number ( $C_{j,k}$ ) in the *P. marinus* population in a specific time interval. This is calculated from the difference in the specific parasite cell division and mortality rates as:

$$\frac{dC_{j,k}}{dt} = (r_d(\rho)_{j,k} - r_m(\rho)_{j,k})C_{j,k} \quad (15)$$

The change in parasite cell number in a time interval,  $\Delta C_{j,k}$ , obtained from equation (15) is converted to calories exchanged between the parasite and its host by:

$$Eg_{j,k} - El_{j,k} = \epsilon V \Delta C_{j,k} \quad (16)$$

where  $\epsilon$  is a conversion factor obtained by assuming that 5 g wet weight is equivalent to 1 g dry weight and that 20 Joules is equivalent to 1 mg dry weight (Laybourn-Parry 1987). Parasite cell volume,  $V$ , is calculated as:

$$V = \frac{4}{3} \pi \left( \frac{D}{2} \right)^3 \quad (17)$$

The average cell diameter,  $D$ , is from Ray (1954a). Coefficient values and definitions are given in Table 3.

The respiratory energy required by the *P. marinus* population is obtained from:

$$Er_{j,k} = \zeta e^{\alpha(T(t)-T_0)} 10^{\omega} V^{\theta} C_{j,k} \quad (18)$$

where the conversion factor,  $\zeta$ , assumes 4.83 mL  $O_2$  per calorie (Powell and Stanton 1985). The exponents  $\omega$  and  $\theta$ , which scale respiration rate to parasite cell volume, are from measurements made for protozoa (Fenchel and Finlay 1983). The value for  $\alpha$  assumes a  $Q_{10}$  of 2 (Laybourn-Parry 1987). The effect of salinity on *P. marinus* respiration rate is unknown and therefore is not included. Coefficient values and definitions are given in Table 3.

#### Effects of Perkinsus marinus on Oyster Physiology

Increased predation is frequently described as a product of parasitism (Jakobsen et al. 1988, Hadelar and Freedman 1989, Schmid-Hempel and Schmid-Hempel 1988). However, no evidence exists for selective predation of *P. marinus*-infected oysters. Thus, selective predation was not included in the model. Also, the possible loss of *P. marinus* during spawning (Dungan and Roberson 1993) is not included. The effects of *P. marinus* infection on oysters that are included are a reduction in oyster filtration rate (Lund 1957) and eventual host mortality.

Mackin and Ray (1955) provide measurements of *P. marinus* that can be used to derive a relationship that describes the reduction in oyster filtration rate with infection intensity. These measurements show an exponential decrease in oyster filtration rate

that depends on the ratio of the number of cells of the parasite to the size (weight) of the host. This reduction in filtration can be expressed as:

$$Dred_{j,k} = \frac{\kappa}{1 + \lambda e^{-\mu \frac{C_i}{W_j}}} \quad (19)$$

Coefficient definitions and values are given in Table 3. The expression given in equation (19), when applied to the oyster filtration rate,  $FR_{T_j}$ , defined in Table 2, results in a fractional reduction in filtration rate as:

$$FR_{D_{j,k}} = FR_{T_j} (1 - Dred_{j,k}) \quad (20)$$

where  $FR_{D_{j,k}}$  is the filtration rate that results when the oysters are infected with *P. marinus*.

The level of *P. marinus* infection in an oyster population is typically diagnosed in terms of a 0-to-5 point scale that was developed by Mackin (1962), with 5 being the heaviest infection level. Field and laboratory measurements show that oyster mortality generally occurs in individuals that have an infection intensity that corresponds to a 5 on this scale (Andrews 1988). Populations with mean infection intensities of 3 or more generally suffer 50 to 75% mortality per year (Ray and Chandler 1955, Mackin 1961, Mackin and Hopkins 1961). These observations provide a basis for determining the lethal *P. marinus* infection level in the simulated oyster populations.

A relationship was developed between host mortality, host size and *P. marinus* cell number by assuming that host mortality occurs when the energy demand of the *P. marinus* population is some fraction of the host's net production. This relationship is based on net production values calculated for uninfected oysters as described by White et al. (1988) and is of the form:

$$\frac{2NP_{w_j}}{Ec} = C_{L_j} \quad (21)$$

where  $NP_{w_j}$  is net production,  $Ec$  is the caloric requirement of the *P. marinus* population as determined from equation (14), and  $C_{L_j}$  is the lethal parasite density (cells oyster<sup>-1</sup>) for any oyster size class,  $j$ .

Equation (21) allows for a size dependency in lethal parasite density that is suggested by measurements given in Choi et al. (1989) and is consistent with a size dependent scope for growth in oyster populations (Hofmann et al. 1992). The factor of 2 used in equation (21) was determined empirically by using yearly mortality rates of 90%, 50% and 10% for the market-size population and comparing the resulting simulated populations with oyster populations reported in the field studies by Ogle and Flurry (1980), Sniat (1985) and Crosby and Roberts (1990).

The lethal parasite density from equation (21) can then be related to oyster size through a regression of the form:

$$C_{L_j} = 10^{(\nu \log_{10} \left( \left( \frac{W_j}{z} \right)^q + \sigma \right))} \quad (22)$$

Note that because equation (22) is obtained from a regression, the units on the two sides of the equation are not equivalent. Coefficient definitions and values are given in Table 3.

Assuming that *P. marinus* infections are initiated by one cell, then, depending on oyster size, 22 to 27 population doublings are needed to reach the lethal density given by equation (22). As required by field observations, equation (22) yields a value of 5 on Mackin's Scale when converted according to Choi et al. (1989) as:

$$C_{L_j} = \nu\tau(10^{6M}) W_j \quad (23)$$

where  $M$  is the Mackin's Scale infection intensity as defined by Craig et al. (1989). Smaller individuals reach lethal infection levels in fewer doublings. Coefficient values and definitions are given in Table 3.

Equation (22) is consistent with the suggestion that oyster mortality could be at least partly explained by a negative energy budget produced when the energy demand of *P. marinus* exceeds the assimilation rate of the oyster (Choi et al. 1989). However, the exact mechanism by which *P. marinus* causes mortality of the oyster host is unknown, and some studies have reported significant effects on the host at lower infection levels (e.g., Paynter and Bureson 1991). The justification for using the approach given by equation (22) comes from favorable comparison between simulated and observed levels of *P. marinus* infection under equivalent environmental conditions.

#### Perkinsus marinus Transmission

The available studies of the transmission of *P. marinus* indicate that oyster density and distance between infected host populations affect the rate of infection (Mackin 1952, Andrews and Ray 1988, Ford 1992), although little information on the transmission of this disease from controlled experiments is available (Andrews 1965, 1988). Therefore, the transmission of *P. marinus* was modeled using general relationships for disease transmission. These formulations were then calibrated against field data.

The specific rate of infection of uninfected oyster individuals,  $r_i$ , was assumed to be the result of an interpopulation transmission rate,  $r_{ib}$ , and an intrapopulation specific transmission rate,  $r_{i0}$  as:

$$r_i = r_{ib} + r_{i0} \left( \frac{P_1 + P_2 + P_3}{3} \right) \quad (24)$$

where  $P_1$ ,  $P_2$  and  $P_3$  are factors that modify the intrapopulation transmission rate.

Insufficient data were available to include the expected relationship between oyster filtration rate and *P. marinus* transmission rate as occurs in other host-parasite systems (e.g., Gee and Davey 1986). This effect could be important at higher latitudes where filtration ceases during the winter, thus limiting transmission rate. However, the decrease in *P. marinus* prevalence and infection intensity produced by the effects of low temperature on parasite growth and mortality, that occur during the winter, should minimize any error due to exclusion of this effect. Also, a suspected influence of salinity on *P. marinus* transmission rate (Paynter and Bureson 1991, Chu and LePeyre 1993) is not included explicitly in the model because of lack of information to adequately parameterize this effect. However, the effect of salinity on parasite density and oyster growth indirectly affects the parasite transmission rate.

The interpopulation infection intensity was determined by using observations from San Antonio Bay, TX obtained as part of the NOAA National Status and Trends program. A catastrophic flood produced 100% mortality of oysters in this bay in 1988. As the bay recovered, the infection intensity and prevalence of *P. marinus* was monitored in the oyster population. These observations showed that *P. marinus* infection returned to regional norms in about 2 years. Simulations of this event required an interpopulation infection intensity ( $r_{ib}$  of  $0.2 \text{ y}^{-1}$ ). Field experiments by Paynter and Bureson (1991) yielded similar results.

Three variables, oyster density, *P. marinus* prevalence and *P. marinus* infection intensity, were used to determine the intrapopulation transmission rate. Factors affecting the intrapopulation transmission rate were formulated as follows. The prevalence of infection in a population varies between 0 and 1, where 0 represents an uninfected population and 1 a population in which all individuals are infected. At each time step in the model the fraction of the total population that was infected with *P. marinus* was calculated and this value was used to specify  $P_1$  as:

$$P_1 = \text{fraction infected.} \quad (25)$$

Mean population infection intensities of 3.5 and above on Mackin's Scale are associated with substantial oyster mortality. Mortality should maximize transmission rate by releasing infective elements into the water column where they are transmitted to other individuals. Thus,  $P_2$  was specified by establishing a ratio between the total cell density,  $TCD$ , in the simulated oyster population and the cell density that corresponds to an infection level ( $IL$ ) of 3.5. Limiting the maximum value to this ratio to 1 yields a maximum transmission rate at all population infection intensities  $\geq 3.5$  as:

$$P_2 = \min \left( 1., \frac{TCD}{IL} \right) \quad (26)$$

where

$$TCD = \frac{\sum_{k=1}^{28} C_k}{\sum_{j=1}^{11} O_{jv}} \quad (27)$$

and  $IL$  corresponds to  $2.5 \times 10^5$  cells (g wet wt) $^{-1}$ .

The proximity of oyster individuals to one another can also affect the rate of disease transmission. This effect is included by comparing the total simulated oyster population density with a relatively high oyster population density ( $OD$ ) and limiting this value to a maximum of 1 as:

$$P_3 = \min \left( 1., \sum_{j=1}^{11} \sum_{k=1}^{28} \frac{O_{j,k}}{OD} \right) \quad (28)$$

where an  $OD$  of 4000 oysters  $\text{m}^{-2}$  is representative of values reported by May (1971) and Dame (1976).

A dense, heavily-infected population [ $(P_1 + P_2 + P_3)/3 = 1$ ] should produce an intrapopulation transmission rate that is capable of infecting all uninfected individuals within six months. To achieve this effect, the maximum intrapopulation transmission rate ( $r_{i0}$ ) was set to  $12 \text{ y}^{-1}$ .

#### Model Implementation

The oyster and *P. marinus* model described above requires input of environmental data such as temperature, salinity, food, turbidity and current flow. Summaries of the environmental time series used in the simulations and the source for each are given in Tables 4 and 5. Concurrent measurements of all of these environmental variables over any time scale for a single estuarine system are rare. Therefore, for this study, time series of these data were obtained from several different environments and combined. The

**TABLE 4.**  
**Environmental time series used as input to the oyster population-*Perkinsus marinus* model.**

<i>Food Supply Time Series (mg l<sup>-1</sup>)</i>											
Dickinson Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0.55	0.50	1.86	1.13	0.92	1.18	0.93	1.92	0.86	1.92	0.91	1.44
April Fools Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0.77	1.21	1.32	1.57	2.15	1.60	1.15	1.50	1.78	1.03	0.45	0.80
Early Spring Bloom (Idealized)											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0.50	0.50	1.25	0.75	0.75	0.75	0.75	0.75	0.75	0.50	0.50	0.50
Late Spring Bloom (Idealized)											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0.50	0.50	0.50	1.25	1.25	0.75	0.75	0.75	0.75	0.75	0.50	0.50
Fall Bloom (Idealized)											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0.50	0.50	0.75	0.75	0.75	0.75	0.75	1.25	1.25	0.50	0.50	0.50
<i>Salinity Time Series (ppt)</i>											
Dickinson Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
2.	10.	6.	8.	9.	13.	16.	18.	24.	23.5	14.	8.
April Fools Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
12.	10.	15.	10.	14.	16.	17.	19.	21.	17.	20.	23.
23.	23.	23.	28.	16.	20.	5.	5.	14.	17.	20.	23.
Biloxi Bay											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
20.	20.	5.	10.	8.	14.	10.	16.	8.	19.	10.	22.
North Inlet											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
34.2	33.9	29.9	29.3	30.2	32.6	33.0	31.1	31.6	34.6	34.2	34.9
<i>Turbidity Time Series (g l<sup>-1</sup>)</i>											
Dickinson Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
.0226	.0265	.0723	.0494	.0496	.0387	.0680	.1461	.0484	.3447	.0399	.0114
April Fools Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
0.049	0.016	0.024	0.074	0.019	0.057	0.016	0.049	0.080	0.088	0.039	0.033
<i>Current Speed Time Series (cm s<sup>-1</sup>)</i>											
Confederate Reef											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
42.	1.	24.	48.	1.	1.	3.	1.	34.	17.	17.	41.
<i>Oyster Abundance—Confederate Reef</i>											
size (upper size limit in mm)											
25.	35.	50.	63.5	70.	76.	88.9	100.	110.	125.	150.	
Abundance (number m <sup>-2</sup> )											
1.8	2.0	4.8	4.6	2.4	1.6	2.2	0.8	0.4	0.2	0.0	

Temperature Time Series are given in Deksheniaks et al. (1993)

Time series are from: Soniat (1985), April Fools Reef, Galveston Bay, Texas; Powell et al. (in press), Dickinson Reef and Confederate Reef, Galveston Bay, Texas; Deksheniaks et al. (1993), Laguna Madre, Texas and Chesapeake Bay; Ogle and Flurry (1980), Biloxi Bay; Crosby and Roberts (1990), North Inlet, South Carolina; Hofmann et al. (1992), idealized spring and fall plankton blooms.

specific combinations of the environmental series for the different simulations are given in Table 5.

In all cases, the environmental time series consisted of monthly-averaged values that extended for one year. The only one of these that requires additional explanation is the time series for current speed that came from Confederate Reef in Galveston Bay, TX (Table 4). These flow measurements were taken 1 to 2 cm above the bottom and are indicative of flow rates experienced by

oyster populations from a moderately-enclosed reef system. Consequently, these speeds may not be representative of the flow rates for some of the bay and estuarine systems used in this study.

The oyster population-*P. marinus* model was solved numerically using an implicit (Crank-Nicolson) tridiagonal solution technique with a one day time step. All simulations began on January 1 (Julian day 1) and ran for 6 years. Each simulation was initialized with an oyster size-frequency distribution obtained from a

TABLE 5.

The combination of environmental time series given in Table 4 and additional parameter values used for the simulations.

Figure Number	Salinity	Temp.	Food	Turbidity	Flow	Halving time	Juvenile Mort.	Comments
	(ppt)			(g l <sup>-1</sup> )	(m s <sup>-1</sup> )	(hours)	(% y <sup>-1</sup> )	
4	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	
6	<i>AF</i>	CB	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	
7	<i>AF</i>	LM	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	
8	<i>AF<sub>m</sub></i>	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	Jan–Feb year 2, 5 ppt
9	30	<i>AF</i>	SL	0.0	0.02	60	99	
9	20	<i>AF</i>	SL	0.0	0.02	60	99	
9	12	<i>AF</i>	SL	0.0	0.10	60	99	larval survival: 1 in 10 <sup>7</sup>
10	<i>AF/DR</i>	<i>AF/DR</i>	<i>AF/DR</i>	<i>AF/DR</i>	<i>CR</i>	60	90	time series split: 2/4
11	30	<i>AF</i>	SE	0.0	0.02	60	99	
11	30	<i>AF</i>	FB	0.0	0.02	60	99	
12	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	no parasite density effect
13	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	80	99	
14	<i>AF/BB</i>	<i>AF/BB</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	30	90	time series split: 2/4
15	<i>AF/NI</i>	<i>AF/NI</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	120	99.7	time series split 2/2
16	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	0% prevalence, set of 20 on Julian day 140
17	<i>AF/NI</i>	<i>AF/NI</i>	<i>AF</i>	<i>AF</i>	<i>CR</i>	60	99	time series split: 1/1/4
18	<i>AF/DR</i>	<i>AF/DR</i>	<i>AF/DR</i>	<i>AF/DR</i>	<i>CR</i>	60	99	time series split: 1/1/4
	<i>/AF</i>	<i>/AF</i>	<i>/AF</i>	<i>/AF</i>				

The different locations for the environmental time series are defined as: April Fools Reef, Galveston Bay, Texas (*AF*), Dickinson Reef, Galveston Bay, Texas (*DR*), Confederate Reef, Galveston Bay, Texas (*CR*), Laguna Madre, Texas (LM), Chesapeake Bay (CB), North Inlet, South Carolina (NI), Biloxi Bay, Mississippi (BB), late spring bloom (SL), early spring bloom (SE), and fall bloom (FB). Time series from Galveston Bay, Texas are indicated by italics. Environmental time series that were modified are designated by *m*. *Perkinsus marinus* division time and juvenile oyster mortality used in each simulation is also shown. Except where indicated, juvenile survival was 1 in 10<sup>8</sup>. Values indicate a 12-month continuous time series at that level.

reef, South Deer Island, in the West Bay section of Galveston Bay, TX in spring 1992 (Fig. 3). The initial density of the individuals in the oyster population was 20 individuals m<sup>-2</sup>. This density and size frequency are representative of Galveston Bay reefs (Powell, unpublished data) and have a mean size that is representative of northern Gulf of Mexico oyster populations (Hofmann et al. 1994). Initially, *P. marinus* was specified to be at 50% prevalence in each oyster size class. This allowed the simulated populations to more rapidly come into equilibrium with environmental conditions than would occur using 0% or 100% prevalence. Initial transients disappeared from the solutions after about one year of simulation.

The simulated distribution of *P. marinus* in the oyster population depends on the rate of larval recruitment and juvenile mortality because new recruits, being uninfected, reduce prevalence and population infection intensity. In most of the simulations, obtaining *P. marinus* prevalence and infection intensities that were comparable to observed values required a larval survivorship of 1 individual in 10<sup>8</sup> larvae spawned and an independent (non-*P. marinus*) source of juvenile mortality yielding a 1% survivorship the first year after settlement. Both survivorship rates are typical of those reported for bivalves (Brousseau et al. 1982, Powell et al. 1984, Cummins et al. 1986). Other survivorship rates were used as indicated in Table 5.

## RESULTS AND DISCUSSION

### Characteristics of Simulated *Perkinsus marinus* Prevalence and Infection Intensity

The first series of simulations were undertaken to ensure that the simulated patterns in *P. marinus* prevalence and infection intensity were consistent with observations from April Fools Reef in Galveston Bay, TX reported by Soniat (1985). Field observations of *P. marinus* (Burrell et al. 1984, Soniat 1985, Crosby and Roberts 1990) show that the prevalence of the disease in the entire oyster population is usually lowest from late winter to early spring. In late spring, as temperatures increase, prevalence rises and peaks in mid to late summer. The pattern seen in *P. marinus* prevalence for the simulated total oyster population (Fig. 4a) shows lows in the summer and highs in the winter, which is the exact opposite of what is observed. The lows in disease prevalence in the simulated oyster population coincide with periods of recruitment following

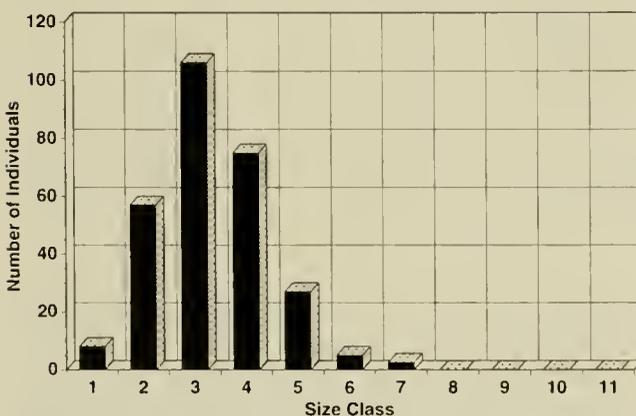
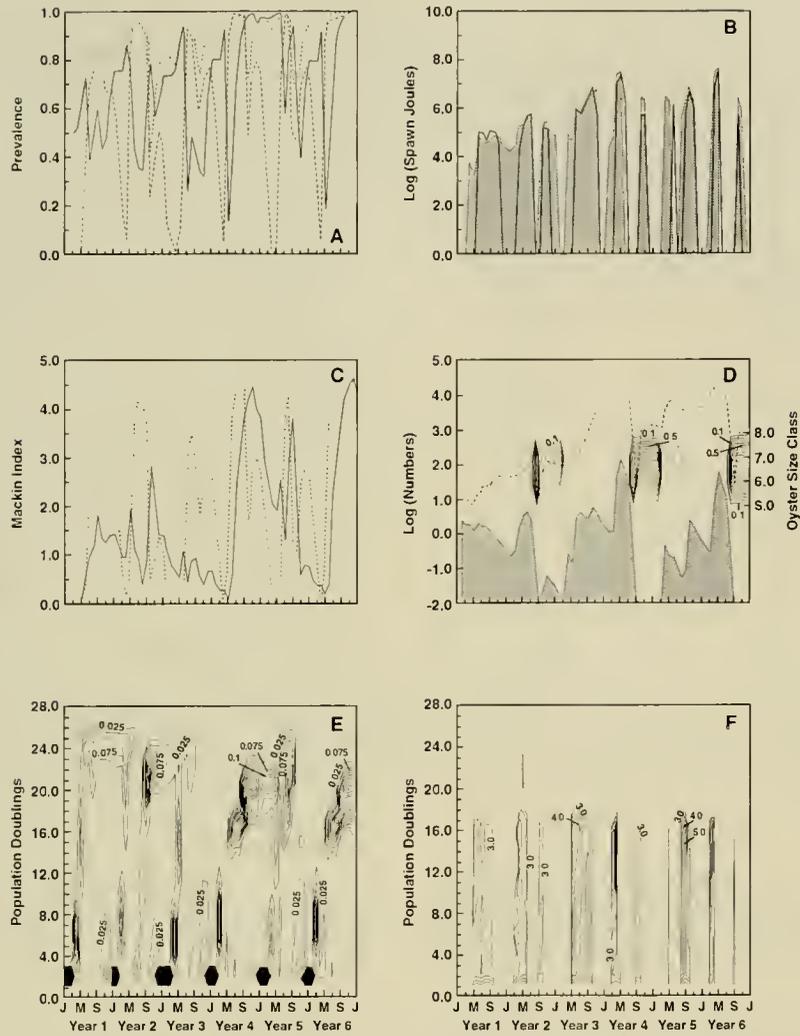


Figure 3. Size-frequency distribution of oysters used to initialize each simulation. Data were obtained at South Deer Island in the West Bay section of Galveston Bay, TX in spring 1992.



**Figure 4.** (A) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, from a simulation that used environmental conditions from April Fools Reef, Galveston Bay, TX (Tables 4 and 5). Prevalence in the entire oyster population, the market-size ( $\geq 3$  inches) portion of the oyster population, and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described by Ray (1966) are represented by the solid, dotted and dashed lines, respectively. (B) Simulated reproductive effort (solid line) for the oyster population in terms of  $\log_{10}(\text{total Joules spawned per month})$ . The quantity of gametic tissue present in the adult oysters, as  $\log_{10}(\text{total Joules present on day 30 of each month})$ , is shown by the shaded region. (C) Simulated oyster population infection intensity expressed in terms of Mackin's Scale. Infection intensity in the entire oyster population and that in the market-size portion of the population are given by the solid and dotted lines, respectively. (D) The number of market-size individuals (solid shading) and submarket-size adults (dashed line) in the population expressed as the  $\log_{10}(\text{number } \text{m}^{-2})$ . Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (E) The fraction of the entire oyster population that is found in each of the 28 *Perkinsus marinus* infection levels. Contour interval is 0.025. (F) Energy spawned ( $\log_{10}(\text{Joules})$ ) by the oysters in each of the 28 levels of *Perkinsus marinus* infection. Contour interval is 0.5.

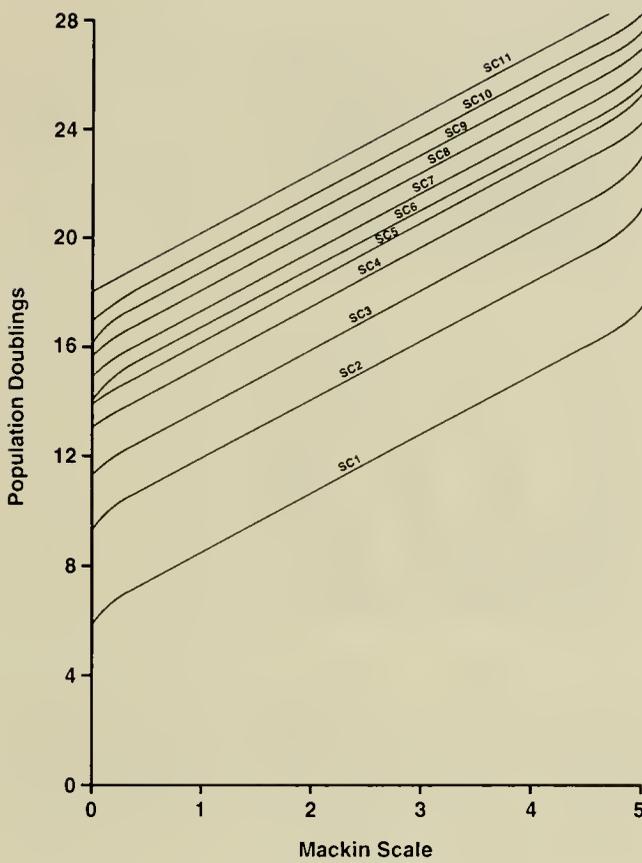
major spawning events (Fig. 4b). Prevalence declines with the addition of uninfected individuals and rises in the fall and winter as these individuals become infected with the disease.

The standard field measurement of prevalence is based upon collection of the largest individuals in the population, which are normally those of market size. When viewed in this way, disease prevalence (Fig. 4a) in the simulated oyster population exceeds 80% in most months but shows lows in the summer and fall as submarket-size adults with lower prevalence grow to market size.

The standard technique for determining *P. marinus* prevalence uses incubation of a slice of mantle tissue in thioglycollate medium (Ray 1966). Choi et al. (1989) showed that very light infections were frequently misdiagnosed as negative on this basis. For ex-

ample, a very light infection, as measured with the standard technique, occurs 7 to 18 doublings after the initiation of infection, assuming a single cell initiates an infection (Fig. 5). Assuming that cell densities of  $\leq 2^{12}$  individual $^{-1}$  are misdiagnosed as negative in the market-size portion of the population yields the normally measured seasonal cycle in prevalence in the simulated population (Fig. 4a). Low prevalence occurs in February and March in most years because infection intensity is low which results in many false negatives being recorded.

Thus, obtaining the normal seasonal cycle in prevalence that is observed in natural oyster populations requires subsampling the simulated populations according to standard field protocol and the inclusion of false negatives at low infection intensities. The same



**Figure 5.** *Perkinsus marinus* population doublings from the initiation of infection by a single cell as a function of the rating given by Mackin's Scale. Each line shows the course of infection for the mean-sized individual in each of the 11 oyster size classes defined in Table 1.

is true of infection intensity. Infection intensity in the entire simulated oyster population, as measured on Mackin's Scale, shows considerable variability (Fig. 4c). Infection intensity varies between 0.1 and 2.0 in the winter and between 1 and 4.5 during the summer and fall. Infection intensity does not always rise in April and May as normally observed, nor are yearly highs always in the summer and early fall and yearly lows during February and March. In comparison, *P. marinus* infection intensity in market-size individuals, selected by the standard field protocol (Fig. 4c), shows the typical seasonal progression of disease with infection highs in May through October and infection lows in February and March. The summer increase in infection in larger individuals is offset by the reduction in infection intensity produced by recruitment of uninfected individuals into the population so that the timing of recruitment significantly affects population infection intensity. The highest population infection intensity (greater than 4 on Mackin's Scale) occurs in year 4 of the simulation when spawning is least (cf. Fig. 4b). Thus, the observed seasonal cycle is dependent upon the size class structure of the sampled population and the standard use of the largest size classes does not always accurately portray infection in the entire population.

The simulated yearly cycle of disease intensification was calibrated against observations from April Fools Reef, Galveston Bay that are presented in Sniat (1985). This data set provides temperature, salinity, turbidity and food observations that are concurrent with observations of *P. marinus* prevalence and infection

intensity. These environmental data sets were used as input to the model (Table 4). The resulting simulated infection intensity (Fig. 4c) averages between 2 and 3 during the summer and fall and between 0.1 and 0.5 in the winter. Infection intensity rises in April and May, reaching yearly highs during the summer and early fall. Yearly lows occur in February and March. Note that Sniat (1985) used a 0-to-6 point scale for infection intensity, rather than the 0-to-5 point scale used for the simulated distributions. Prevalence in the simulated populations averages between 50 and 90% during the summer and drops to between 0 and 10% in the winter (Fig. 4c). These results agree favorably with the observations given in Sniat (1985), except that the prevalences reported by Sniat (1985) average somewhat lower in the summer and somewhat higher in the winter. This discrepancy between the simulated and observed prevalences could result from uncertainties in the sampled size-frequency distributions, the accuracy of detection of very light infections, or inaccuracies in model parameters. Direct comparisons of simulated and observed prevalences can be further confounded because the portion of the simulated oyster population experiencing mortality (Fig. 4d) or infection from *P. marinus* (Fig. 4e) can vary seasonally and interannually.

#### *Factors Controlling Prevalence and Infection Intensity*

The prevalence and infection intensity of *P. marinus* in oyster populations is controlled by environmental and biological processes. Environmental regulation is exerted through variations in temperature, salinity and food supply. Biological controls are from density effects on the rate of parasite division, and parasite and host mortality. Additionally, recruitment of uninfected individuals to the oyster population and subsequent transmission of the disease to these individuals alters the observed prevalence and infection intensity of the disease. Each of these is examined in the simulations described below.

#### *Temperature*

The primary influence of increasing temperature over a seasonal cycle is to increase the rate of parasite division and decrease the rate of parasite mortality through lowered activity of the oyster's defense system. Both effects work to intensify *P. marinus* infection. However, such a simple characterization of the influence of temperature cannot be extrapolated across latitudes because changing temperatures interact with numerous physiological processes that affect oyster populations. Thus, the effects of temperature were examined using temperature time series from Chesapeake Bay and the Laguna Madre of Texas as well as from Galveston Bay (Table 4). Summer temperatures are similar across this latitudinal range. However, average winter temperatures are much colder in Chesapeake Bay and the spring rise and autumn fall in temperature occurs later and earlier, respectively, in Chesapeake Bay than in the Laguna Madre (Deksheniaks et al. 1993).

Comparison with observations from the NOAA Status and Trends program (Table 6) shows that the simulated winter prevalences and infection intensities of *P. marinus* in Chesapeake Bay (Fig. 6) are similar to observed values. Also, the simulated yearly cycle is similar to that described by Andrews (1988). The simulated prevalence of *P. marinus* in Chesapeake Bay peaks during the summer as it does in Galveston Bay (Fig. 6a vs. 4a). Winter prevalence in Chesapeake Bay, however, is characterized by values below 10% for up to 5 months during the winter and early spring. *P. marinus* infection intensity averages lower in Chesa-

TABLE 6.

*Perkinsus marinus* prevalence and infection intensity for sites sampled along the east coast of the United States in 1988.

Site	Prevalence	Median Infection Intensity	Confidence 5%	Limits 95%
Delaware Bay, Kelly Island	5.0	0.00	0.00	0.00
Delaware Bay, False Egg Island Point	5.0	0.00	0.00	0.00
Delaware Bay, Ben Davis Point Shoal	0.0	0.00	0.00	0.00
Delaware Bay, Arnolds Pt. Shoal	6.7	0.00	0.00	0.00
Chesapeake Bay, Dandy Point	30.0	0.00	0.00	0.00
Chesapeake Bay, Hackett Point Bay	0.0	0.00	0.00	0.00
Chesapeake Bay, Mt. Point Bar	0.0	0.00	0.00	0.00
Chesapeake Bay, Hog Point	0.0	0.00	0.00	0.00
Quinby Inlet, Upshur Bay	25.0	0.00	0.00	0.00
Roanoke Sound, John Creek	25.0	0.00	0.00	0.00
Pamlico Sound, Wysoching Bay	38.3	0.00	0.00	0.00
Cape Fear River, Battery Island	100.0	1.00	0.67	1.00
Charleston Harbor, Shutes Folly Island	68.3	0.33	0.33	1.00
Charleston Harbor, Fort Jackson	45.0	0.00	0.00	0.33
Savannah River Estuary, Tybee Is.	80.0	0.33	0.33	1.00
Sapelo Sound, Sapelo Island	90.0	1.00	0.33	1.00
St. Johns River, Chicopit Bay	91.7	1.00	1.00	1.00
Matanzas River, Crescent Beach	93.3	1.00	1.00	1.00
Indian River	97.5	1.33	1.00	1.67
North Miami	93.3	1.00	1.00	1.00

The sampling was done as part of the NOAA Status and Trends ("Mussel Watch") Program. Site descriptions are given in Boehm et al. (1988). Prevalence was computed as the fraction of infected individuals and infection intensity was assayed by the thioglycollate method (Ray 1966) and rated from 0 to 5 on the scale given in Mackin (1962) and modified by Craig et al. (1989). Confidence limits for the median values were calculated by bootstrapping.

peake Bay than in Galveston Bay (Fig. 6b vs. 4c) during the entire year. Also, the simulated cycle of increasing and decreasing infection intensity is more regular year-to-year in Chesapeake Bay and the winter lows in infection intensity begin earlier in the fall and extend later into the spring, typically from November to April. The later increase in prevalence and infection intensity in the spring in Chesapeake Bay as compared to Galveston Bay is due to the cooler spring temperatures. The rapid drop in the simulated prevalence and infection intensity is due to the drop in fall temperature and to the recruitment of uninfected individuals, which is temporally constrained in Chesapeake Bay due to a more restricted spawning season (Dekshenieks et al. 1993, Hofmann et al. 1994). Finally, in the Chesapeake Bay simulation, *P. marinus* produces less mortality during the summer months than in Galveston Bay although declines in market-size individuals in July and August do occur (Fig. 6c).

Warmer temperatures characteristic of the Laguna Madre yield simulated summer prevalences of *P. marinus* that are similar to Galveston Bay. However, winter prevalences are higher (Fig. 7a vs. 4a). Infection intensities in the Laguna Madre average lower during the summer (Fig. 7b vs. 4c). These trends are in agreement with measurements made as part of the NOAA Status and Trends program in the Laguna Madre (Craig et al. 1989, Wilson et al. 1990). However, significant *P. marinus*-induced summer mortality events occur in the Laguna Madre (Fig. 7c vs. 4d) which limit the market-size component of the population, and, in fact, may be important in restricting the characteristic adult size of the population. Simulations of the oyster populations in Laguna Madre show that characteristic adult size of these populations averages smaller than would be expected simply from the effect of temperature on growth and reproduction (Hofmann et al. 1994).

As in the Galveston Bay simulation, the population prevalence and mean infection intensity of *P. marinus* in the Laguna Madre fail to be described adequately by the standardly-collected data obtained from the larger oyster size classes. Recruitment of uninfected individuals in the summer produces population lows that are coincident with highest prevalences and infection intensities in the market-size component. *P. marinus*-induced mortality events in the large size classes in the summer and early fall produce lower infection intensities in the winter in the largest size classes than observed in the entire population. Also, an extended reproductive season in Galveston Bay and the Laguna Madre results in the recruitment of uninfected individuals sporadically over much more of the year, which serves to lower disease prevalence and intensity in the entire population. Only for the Chesapeake Bay simulation do the measures of *P. marinus* prevalence and infection intensity observed in the entire population agree reasonably well with the normally-assayed market-size population. In this bay, the shorter spawning season results in the introduction of uninfected individuals in phase with the late summer mortality events and the decline in infection intensity that comes with cooler fall temperatures.

The basic influence of temperature, then, is to promote high summer prevalences and infection intensities of *P. marinus* at mid-latitudes, the northern Gulf of Mexico and much of the southeastern U.S. coast (Table 6; Craig et al. 1989, Wilson et al. 1990), to modify the time span and degree of the winter lows in infection intensity, and to vary the phasing of recruitment and the fall decline in infection intensity. The temperature-controlled progression of infection through the seasons seen in the Chesapeake Bay simulation is less evident for Galveston Bay and difficult to discern in the Laguna Madre in all but the market-size component of the population. Therefore, the regularity of infection increase and

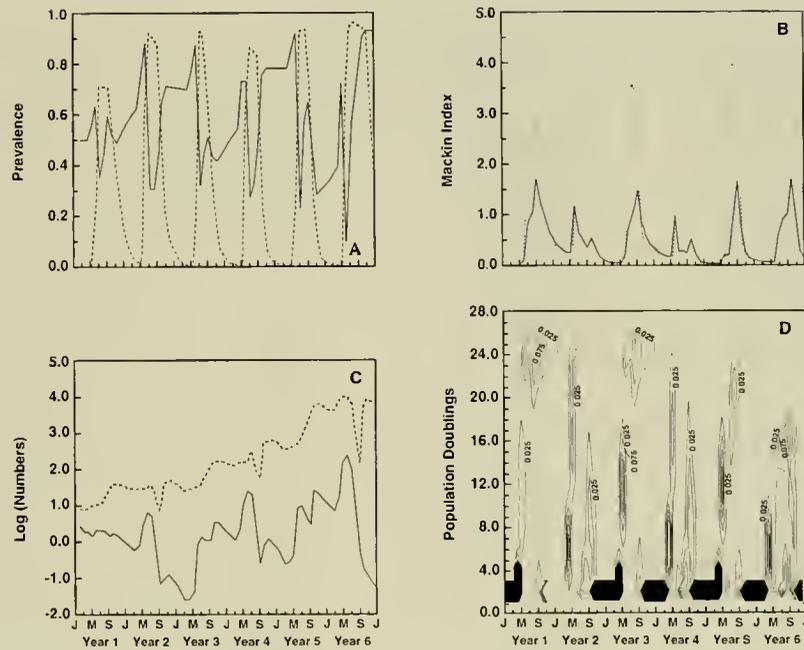


Figure 6. (A) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, from a simulation that used environmental conditions from Chesapeake Bay (Tables 4 and 5). Prevalence in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (B) Simulated oyster population infection intensity expressed in terms of Mackin's Scale. Infection intensity in the entire oyster population and in the market-size portion of the population are given by the solid and dotted lines, respectively. (C) The number of market-size individuals (solid line) and submarket-size adults (dashed line) in the population in terms of  $\log_{10}(\text{number } \text{m}^{-2})$ . (D) The fraction of the entire oyster population that is found in each of the 28 *Perkinsus marinus* infection levels. Contour interval is 0.025.

decrease with the seasons seen in the simulated oyster populations corresponding to northern climes results from the strong temperature effect on *P. marinus* growth and mortality and the restricted duration of the spawning season. As a result, the course of infection varies from a regularly periodic phenomenon in northern climes to a more variable procession in southern climes. The tendency for heavy infections to persist into the winter months in the simulated oyster populations is greatly increased and mortality rises in southern climes under otherwise similar environmental conditions. However, the simulations suggest that *P. marinus* mortality as a fraction of the adult oyster population may be most important at mid-latitudes such as the northern Gulf of Mexico in most years. Increased reproductive potential at lower latitudes balances the tendency towards increased rates of mortality from disease. Consequently, infection intensities and prevalences average higher in the central part of the latitudinal range of *P. marinus*. Unfortunately, no experimental analyses of latitudinal variations in the yearly cycle of *P. marinus* are available for comparison with the simulated distributions. Hence, verification of this suggested effect must await field measurements.

#### Salinity

As shown in Figure 2c, the coincidence of low salinity with high temperature results in mortality of *P. marinus* and hence a reduction in infection intensity. The second year of the salinity time series from April Fools Reef (Table 4) contains a low salinity event in July and August. Soniat (1985) reported a reduction in *P. marinus* infection intensity in the oyster populations in Galveston Bay that corresponded to this event. The simulations using this salinity time series show a decline in disease prevalence and infection intensity in years 2, 4 and 6 (Fig. 4c). Although the degree

of decline is abetted by recruitment, growth and adult mortality which simultaneously reduce infection intensity, the results of this simulation agree with the field observations presented in Soniat (1985).

The insertion of a second equivalent low salinity event in January and February in year 2 of the April Fools Reef salinity time series gives the simulated infection intensities shown in Figure 8. The winter low salinity event had little effect on infection intensity, nor was prevalence impacted to a significant degree. These results agree with field observations and the experimental data given by Fisher et al. (1992).

To examine the impact of varying salinity regimes on the course of infection, simulations were run with a constant salinity of 30 ppt, 20 ppt and 12 ppt (Fig. 9). At 30 ppt, prevalence and infection intensity were relatively unchanged from that at 20 ppt (Fig. 9a); however, oyster mortality was much higher (Fig. 9b vs. 9c). Simulations of oyster populations (Hofmann et al. 1992) indicate that salinity values between 20 and 30 ppt have no appreciable effect on oyster physiological processes, except as mediated by *P. marinus* through the balance between parasite growth and mortality. Thus, the increase in mortality at the higher salinity is due to *P. marinus*.

High salinity is frequently identified as a triggering mechanism for epizootics of *P. marinus*. This simulation shows the substantial impact of disease at high salinity on mortality in the market-size portion of the population. However, these simulations show that, even at high salinity, an oyster population is resistant to a population crash induced by *P. marinus* as long as recruitment of uninfected individuals continues. This mechanism is believed to sustain the oyster populations on Confederate Reef in Galveston Bay (Powell et al. 1992). The reports of epizootic events in oyster

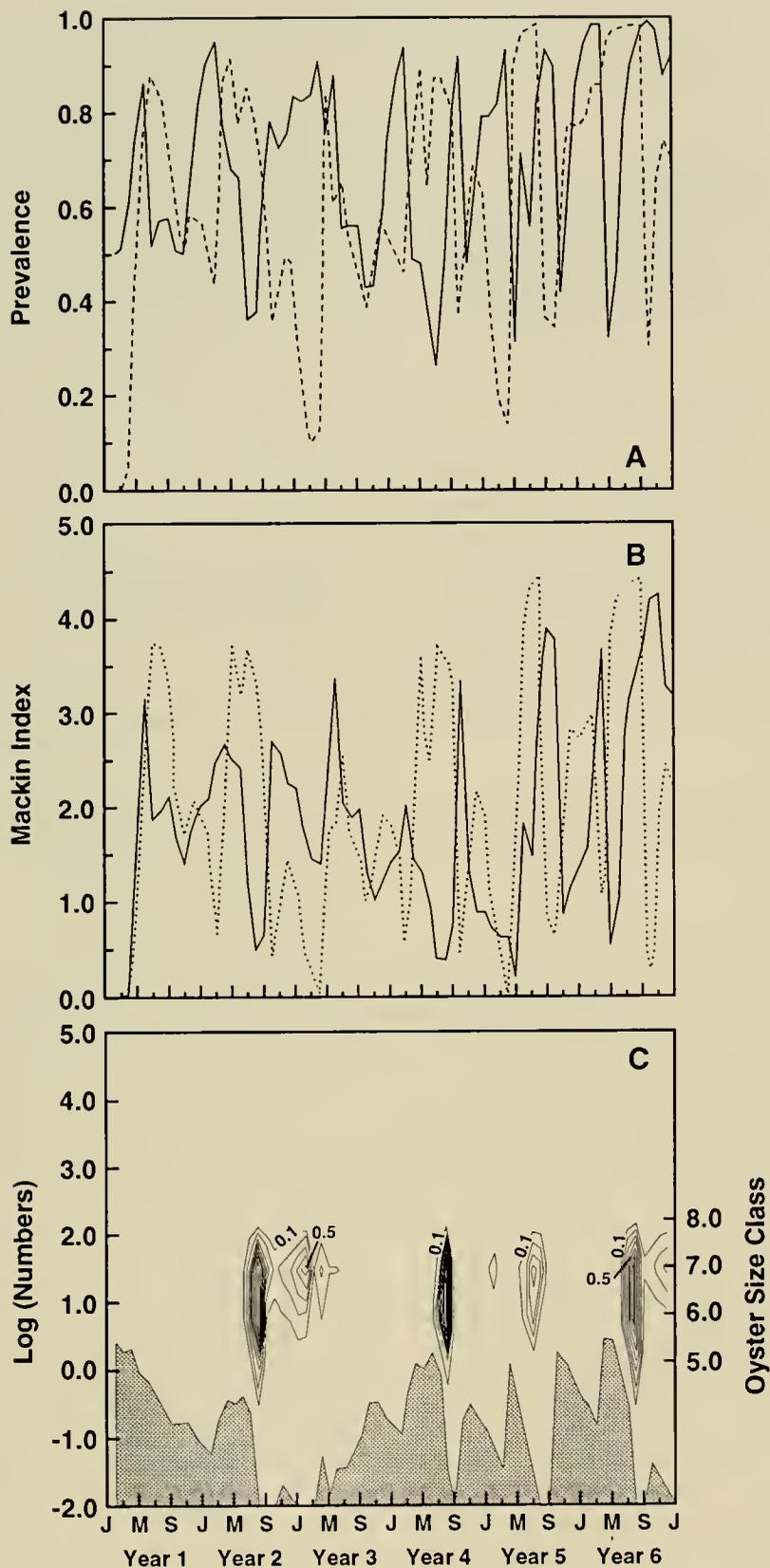


Figure 7. (A) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, from a simulation that used environmental conditions from Laguna Madre, TX (Tables 4 and 5). Prevalence in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (B) Simulated oyster population infection intensity expressed in terms of Mackin's Scale. Infection intensity in the entire oyster population and in the market-size portion of the population are given by the solid and dotted lines, respectively. (C) The number of market-size individuals (solid shading) in the population in terms of  $\log_{10}(\text{number m}^{-2})$ . Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1.

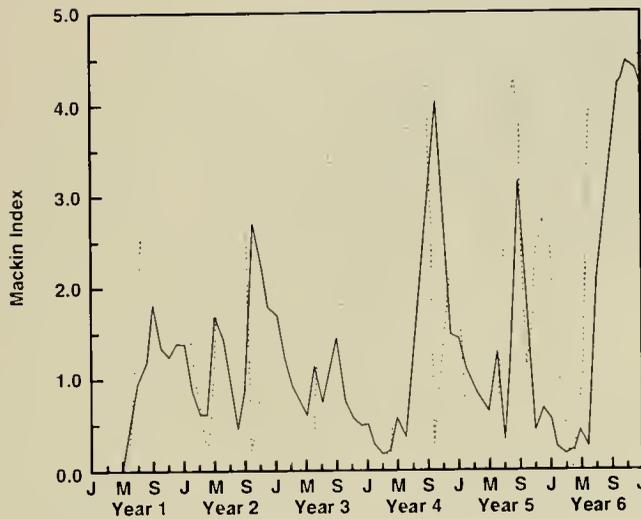


Figure 8. *Perkinsus marinus* infection intensity expressed in terms of Macklin's Scale in a simulated oyster population from Galveston Bay that has been subjected to low salinity events in January–February and July–August of years 2, 4 and 6 (Tables 4 and 5). Solid line indicates infection intensity in the entire population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the oyster population.

populations due to *P. marinus* include only one in which a simultaneous recruitment event occurred (May 1971). Thus, a rise in salinity alone may not be sufficient to generate an epizootic of *P. marinus* in oyster populations.

A constant salinity of 12 ppt results in the demise of the oyster population. Infection intensity declines in the market-size portion of the population, as expected (Fig. 9d) and no *P. marinus*-induced mortality occurs. However, the expected benefit of reduced infection intensity is offset by a decline in reproductive output (Fig. 9e). The reduction in reproductive output results from the higher maintenance cost incurred at lower salinity. This, coupled with the persistent energy demand of *P. marinus*, results in reduced fecundity (Fig. 9e). Simulations of oyster populations (Hofmann et al. 1992) show that the effects of an increased maintenance requirement at lower salinity can be offset by increased food supply. Also, decreased juvenile mortality would offset the effects of reduced fecundity. This simulation suggests that the beneficial effects of low salinity in reducing the infection intensity and prevalence of *P. marinus* are useful only when combined with other factors, such as increased food. As has been reported for Chesapeake Bay (Andrews 1988, Ragone and Burreson 1993), *P. marinus* is capable of surviving and generating significant infection levels at low salinity.

#### Food Supply, Growth and Reproduction

The effect of variations in food supply and turbidity on *P. marinus* prevalence and infection intensity was tested by constructing a time series in which food supply was adequate for 2 years and then decreased for the following 4 years. This was done by combining food concentration measurements from April Fools Reef and Dickinson Reef in Galveston Bay (Table 5). The Dickinson Reef values were collected about 10 years later than the April Fools Reef values (Powell et al. in press). Turbidity and food values in the Dickinson Reef time series average higher and lower, respectively relative to the values for April Fools Reef. Temper-

ature and salinity conditions are similar at the two locations, which are about 1 kilometer apart.

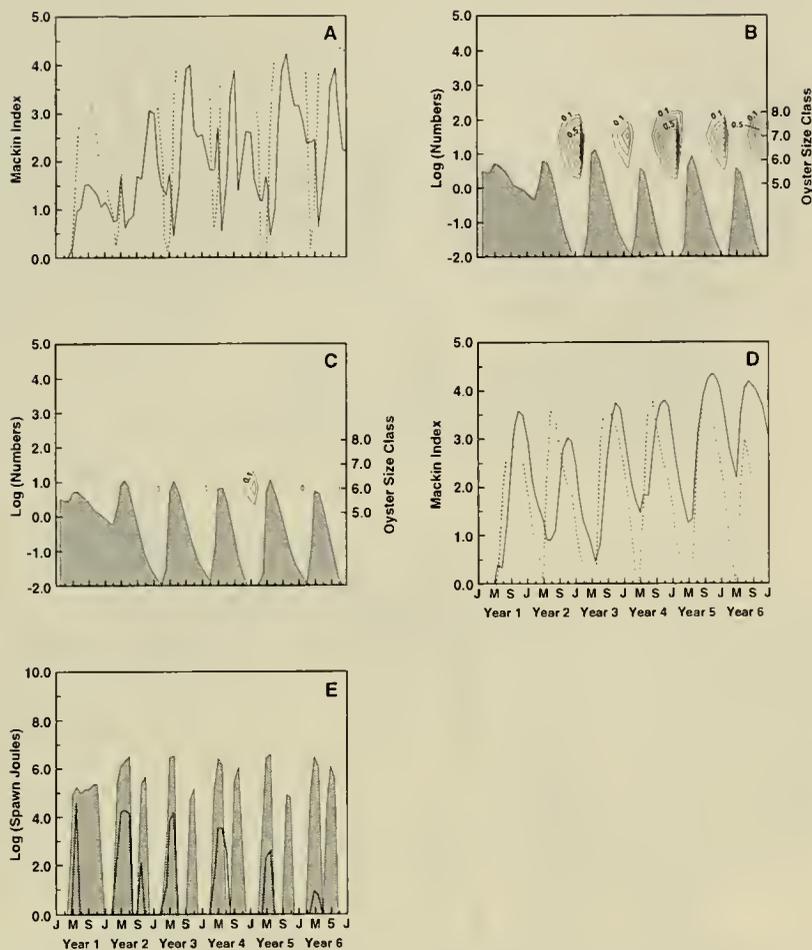
The combination of the environmental time series in this manner significantly alters the oyster population structure. An initial healthy and growing oyster population, with a substantial market-size component, begins a steady decline after exposure to the higher turbidity and lower food concentrations, which reduce oyster ingestion rate. The market-size component of the population steadily declines after year 2 of the simulation (Fig. 10a). The simulation shows that a restriction in food supply can mimic the effect of overfishing in that the market-size component of the size-frequency distribution is selectively lost from the population.

The spawning potential of the oyster population is not significantly impacted until year 6 (Fig. 10b), so that the population decline is not due initially to a significant decrease in fecundity. The population decline is produced by a series of *P. marinus*-induced mortality events which occur each summer during periods of reduced food supply (Fig. 10a). A dramatic increase in *P. marinus* infection intensity occurs in the spring of years with reduced food supply and then drops in July and August in the market-size population as mortality removes most of the heavily infected portion of the population (Fig. 10c). The restriction in food supply also changes the nature of the fall-winter decline in disease intensity (Fig. 10b). More heavily infected individuals are retained in the population over winter because a restricted food supply results in a negative energy budget that reduces oyster biomass. This maintains a high *P. marinus* density that then inhibits parasite mortality. In the later years of the simulation, oyster mortality begins earlier in the spring as these individuals begin to die (Fig. 10a).

Simulations presented in Hofmann et al. (1992) show that the timing of maxima in food supply, as occurs in spring and fall phytoplankton blooms, is important in determining the growth and fecundity characteristics of oyster populations. Thus, an interaction between host food supply and *P. marinus* should be expected. As the timing of the phytoplankton bloom moves through the year, from March–April (Fig. 11a) to April–May (Fig. 9b) and then to August–September (Fig. 11b), the level of *P. marinus*-produced mortality in the market-size fraction of the simulated oyster population changes dramatically. An early spring bloom produces less mortality because a larger fraction of the net production goes into somatic growth, and one of the primary defenses for the oyster is to outgrow the disease.

Infection intensity in the market-size population is markedly different with the fall bloom (Fig. 11d) as compared to either an early or late spring bloom (Fig. 11c, 9a). A secondary minimum occurs in the summer due to the growth of uninfected individuals to market-size. Moreover, infection intensity in the entire oyster population tracks that in the market-size individuals less and less well as the time of maximum food supply moves through the seasons. This suggests that an increasingly biased view of the disease is possible using standard sampling protocols, which emphasize market-sized adults, as food becomes available increasingly later in the year. However, there are no data available against which this result can be compared.

Simulations for Chesapeake Bay provide a similar sequence of events as long as the bloom is approximately phased with the spring rise or fall decline in temperature. Hence, the result of simulations of Chesapeake Bay with an April–May bloom are similar to those obtained for Galveston Bay with the bloom one month earlier.



**Figure 9.** (A) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale in a simulated oyster population that is subjected to a constant salinity of 30 ppt. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population. (B) The number of market-size individuals (solid shading) in the population in terms of  $\log_{10}(\text{number m}^{-2})$ . Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (C) The number of market-size individuals (solid shading) in the population in terms of  $\log_{10}(\text{number m}^{-2})$  in a simulated oyster population exposed to a constant salinity of 20 ppt. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (D) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale in a simulated oyster population that is subjected to a constant salinity of 12 ppt. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population. (E) Simulated reproductive effort for the oyster population in terms of  $\log_{10}(\text{total Joules spawned per month})$  for the oyster populations at 20 ppt (solid line) and at 12 ppt (shaded region).

Juvenile oysters generally have low infection intensities (Ray 1954b), which are believed to be the result of the relative growth rates of host and parasite (Mackin 1951). These oysters grow fast enough that they can reach reproductive size (submarket-size adults) before the disease can intensify. A lower infection intensity in submarket-size adults in the simulated populations is dependent upon a combination of factors including relatively slow rates of disease transmission over the winter, the time required for the disease to intensify during the winter and early spring, and the timing of initial recruitment. Recruitment to the simulated populations in the late summer provides a longer growing season for juveniles at lower infection levels because they acquire the disease near the time that the fall decline in infection intensity begins, and thus their mean infection intensity remains low. A reduction in food supply reduces growth and therefore promotes higher infection intensity. Furthermore, the combined effect of reduced food supply and the subsequent increase in disease intensity should

significantly reduce population fecundity. As a result, recruitment ultimately declines. Finally, intensification of disease reduces filtration rate, which will magnify the effects of a decreased food supply. Thus, this host-parasite system should be very sensitive to variations in host food supply as shown in Figures 9 to 11.

*P. marinus* is thought to restrict oyster fecundity (Mackin 1952, Wilson et al. 1988, Choi et al. 1993, 1994). The simulations consistently show that oyster populations with higher infection intensities fail to spawn (Fig. 4f). Interestingly, these include all oysters having light-moderate or higher infection intensities, which indicates the sensitivity of oyster reproduction of *P. marinus* infection intensity. Decreased reproduction is a standard result of parasitism (e.g., Anderson 1977, Blower and Roughgarden 1987, Festa-Bianchet 1989), but this effect has been difficult to quantify for *P. marinus* (Choi et al. 1994). The simulations suggest one possible reason for this. More heavily-infected individuals have delayed spawning, and at the higher end of the infection

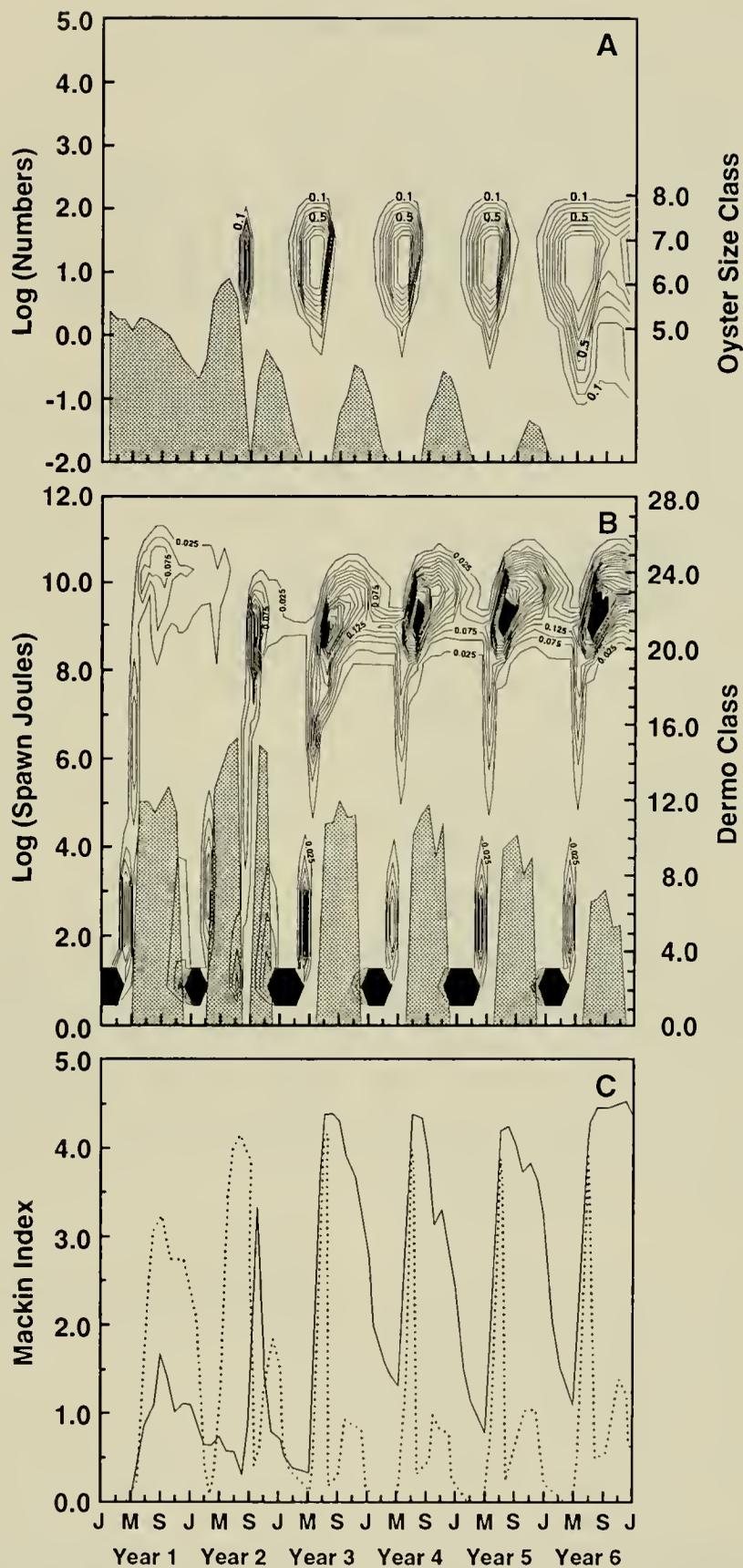


Figure 10. (A) The number of market-size individuals (solid shading), expressed as  $\log_{10}(\text{number m}^{-2})$ , for a simulated oyster population from Galveston Bay, TX that is subjected to a declining food supply in the last four years of the simulation as described in Tables 4 and 5. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (B) Reproductive effort of the simulated oyster population (shaded region) in  $\log_{10}(\text{total Joules spawned per month})$  and the distribution of oysters among the 28 *Perkinsus marinus* infection levels. Contour interval is 0.025. (C) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population.

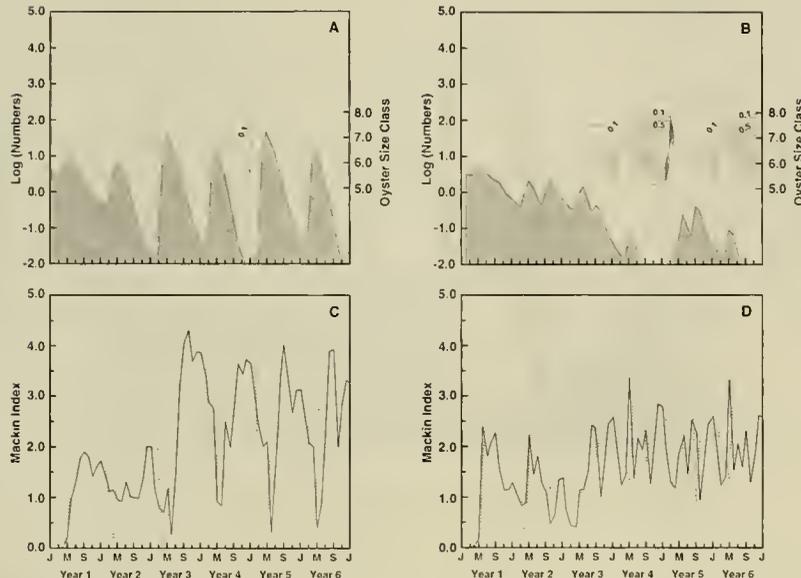


Figure 11. (A) The number of market-size individuals (solid shading), expressed as  $\log_{10}(\text{number m}^{-2})$ , for a simulated oyster population exposed to high food supply in early spring (March–April). Other environmental conditions are for Galveston Bay, TX as described in Tables 4 and 5. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (B) The number of market-size individuals (solid shading), expressed as  $\log_{10}(\text{number m}^{-2})$ , for a simulated oyster population exposed to high food supply in late summer (August–September). Other environmental conditions are for Galveston Bay, TX as described in Tables 4 and 5. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (C) *Perkinsus marinus* infection intensity expressed in terms of Mackin’s Scale for the simulated oyster population shown in Panel A. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population. (D) *Perkinsus marinus* infection intensity expressed in terms of Mackin’s Scale for the simulated oyster population shown in Panel B. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population.

scale, little spawning occurs at all. However, these animals have gametes; they simply fail to spawn. A standard histological analysis might fail to detect this effect (Choi et al. 1993). A relationship between the rate of oyster gamete protein production and *P. marinus* infection intensity has been observed, but only in months when disease intensity is high (Choi et al. 1994). Hence, verification of the interaction between disease and spawning suggested by the simulations must await field observations.

**Parasite Density**

*P. marinus* cell division and mortality rates decline at high parasite densities. When the density effect on parasite division rate is not included in the model, the simulated course of the disease is markedly aberrant (Fig. 12). Oyster population mortality rate is dramatically higher (Fig. 12a vs. 4d), infection intensities are lower and the change from decreasing to increasing intensity is more abrupt (Fig. 12b). Low infection intensity is maintained by the high rate of *P. marinus*-induced mortality in the oyster population. The long period of high infection intensities typically observed in the summer fails to occur (Fig. 12b vs. 4c). Thus, these simulations without a parasite density effect fail to agree with observations (e.g., Soniat 1985) in either magnitude or in form. The plateau of high infection intensity during the summer seen in all *P. marinus* data sets (e.g., Soniat 1985, Crosby and Roberts 1990, and others referenced herein) requires a decrease in parasite division rate at high parasite density and a reduction in the rate of parasite mortality. Without this effect, oysters would require less than 1 week to progress from a moderately-heavy to a lethal infection, because a moderately-heavy infection is only a few population doublings away from death (Fig. 5).

The simulations suggest that the cap on oyster population infection intensity in the range of 3 to 4 on Mackin’s Scale (moderate to moderately-heavy) that is observed in oyster populations that have high disease prevalence results from two processes. First, reduced rates of parasite division and parasite mortality allow oysters to remain moderately to moderately heavily infected for extended periods of time. Second, the rate at which these individuals reach a lethal infection level is slow enough that less heavily-infected oysters of submarket size can grow up to replace those that die. Growth rates of submarket-size adults and parasite division rates in moderately-heavy infected individuals are similar. Thus, in the simulated oyster populations infection intensity is buffered during the summer in the range of 3 to 4 on Mackin’s Scale by the effect of parasite density on its rate of division and mortality.

The density effect on parasite division serves to retain infection in oyster populations since parasite death (by oyster death) is also minimized. High host infection rates do not necessarily result in high host mortalities in contrast to many host-parasite systems (e.g., Anderson and Gordon 1982, Dobson and Hudson 1992, Hudson et al. 1992). For example, with Galveston Bay conditions, oysters are heavily infected (doublings 20 to 28) in the summer and lightly infected in the winter (doublings 1 to 4). However, in nearly every winter, a portion of the simulated oyster population retains a high infection level (Fig. 4e). Andrews (1988) described this same phenomenon for Chesapeake Bay oyster populations. Mean infection intensities higher than median infection intensities in the Gulf of Mexico during the winter indicate a similar winter situation (Powell, unpublished data). In the simulated distributions no such effect occurs when the effect of parasite density is not

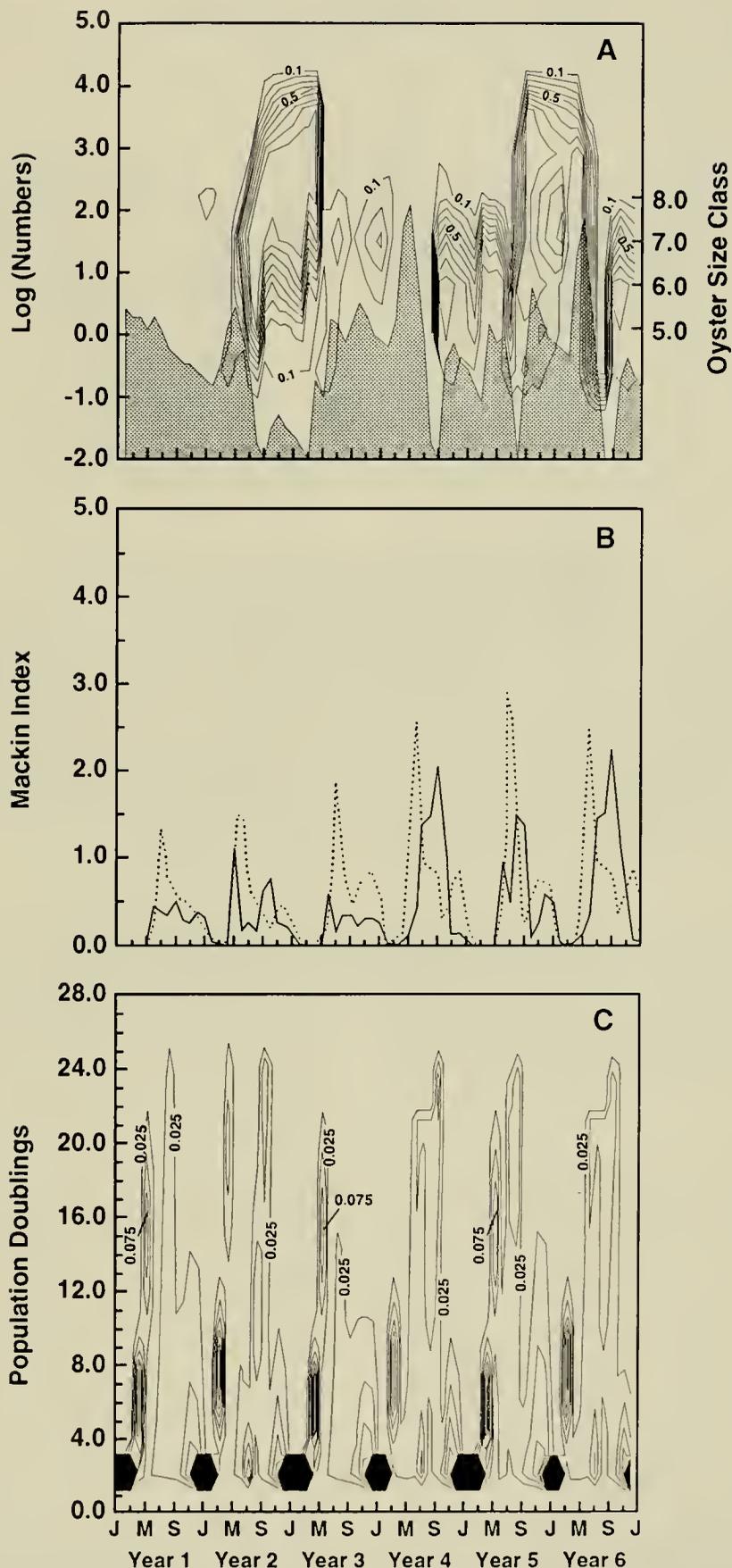


Figure 12. (A) The number of market-size individuals (solid shading), expressed as  $\log_{10}(\text{number m}^{-2})$ , for a simulated oyster population without the effect of *Perkinsus marinus* density on the rate of parasite division or parasite mortality. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (B). *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population. (C) The fraction of the entire oyster population that is found in each of the 28 *Perkinsus marinus* infection levels. Contour interval is 0.025.

included (Fig. 12c), indicating that this effect is the basis for these observations.

The temperature decrease in late fall results in a higher rate of parasite mortality than parasite division. However, because high parasite density slows parasite mortality, the first few halvings occur slowly. As temperature continues to decrease, the rate of parasite mortality slows. The rate of parasite division slows more rapidly, so that infection intensity drops in the fall and winter in most of the population. Eventually the combination of temperature and parasite density cause the rate of parasite mortality to become very low at high infection intensities. Some oysters may retain high infection intensities well into and sometimes throughout the winter months by this mechanism.

Andrews (1988) notes that many of these oysters die during the winter. In the simulations from Galveston Bay (Fig. 4d), mortality events do occur in January and February of years 3 and 5. In the Chesapeake Bay simulations (Fig. 6d), fewer individuals remain heavily infected in the winter as compared to Galveston Bay (Fig. 4e) or the Laguna Madre. These simulations suggest that winter mortality of heavily-infected oysters should be most significant at lower latitudes. However, no direct evidence exists to support or refute this result.

**Mortality**

Oyster mortality occurs at high densities of *P. marinus* when the rate of parasite division exceeds oyster growth rate. A negative energy budget in the oyster abets this process and environmental factors such as salinity and food supply can produce a short-term energy deficit. The role of the rate of *P. marinus* mortality in determining infection intensity is illustrated by the simulation shown in Figure 13 where the halving time at 20°C and 20 ppt (Fig. 2b) of the parasite population was set at 80 hours rather than the normal 60 hours. Infection intensity for the market-size portion of the oyster population remains remarkably stable (Fig. 4c vs. 13a) because increased mortality removes the most heavily-infected individuals from the population and buffers any change in infection intensity (Fig. 13b). However, the reduction in parasite mortality rate changes a stable population of market-size adult oysters into one that is declining over time (Fig. 4d vs. 13b). A proportional increase in the rate of parasite division would, of course, have produced a similar, though not identical, result.

For the simulated prevalences and infection intensities to agree with observations from different bay systems, either the rate of *P. marinus* division or the rate of parasite mortality had to be varied. Many water quality factors can affect the defense system of the oyster (Larson et al. 1989, Fisher et al. 1990, Anderson et al. 1992a); whereas, the impact of water quality on the rate of *P. marinus* division is less well understood (Ray 1965). Thus, the rate of parasite mortality was varied to permit the simulations to agree with observations. With this modification, simulations were done using observations from Biloxi Bay, MS (Ogle and Flurry 1980) and North Inlet, SC (Crosby and Roberts 1990). Both data sets had salinity and temperature time series, but no food or turbidity measurements. Therefore, the food time series reported in Soniat (1985) was used for these simulations. The simulations were done using the first 2 years of the temperature and salinity time series for April Fools Reef and then substituting the appropriate time series from either Biloxi Bay or North Inlet for the remaining years (Table 5).

In Biloxi Bay, salinity is lower than in Galveston Bay, while

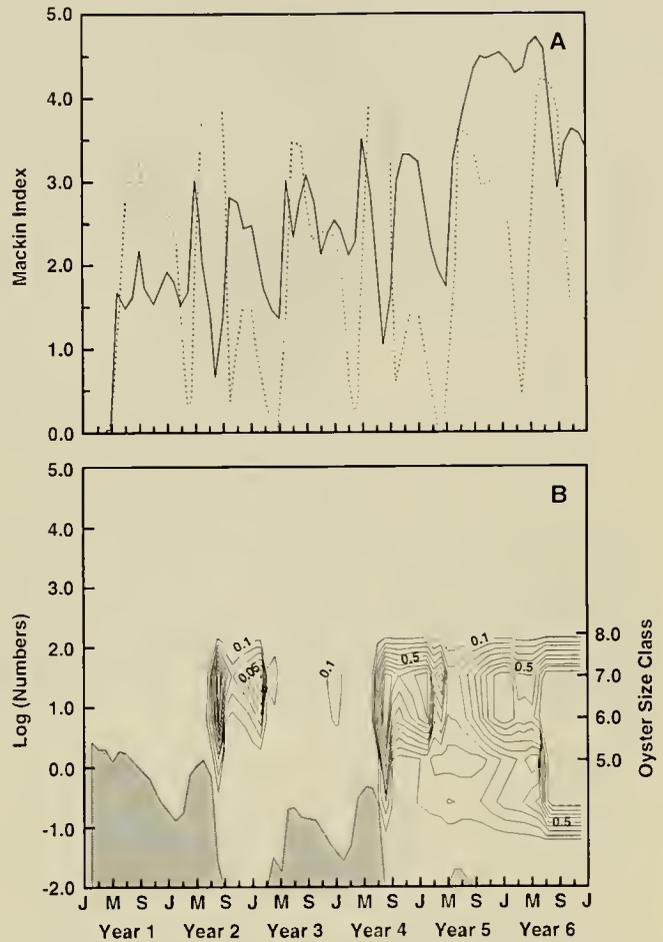


Figure 13. (A) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale for a simulated oyster population in which the parasite halving time was set at 80 hours. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size (≥3 inches) portion of the population. (B) The number of market-size individuals (solid shading) in the population in terms of  $\log_{10}(\text{number m}^{-2})$ . Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1.

temperatures are similar. *P. marinus* prevalence and infection intensity are lower than observed at April Fools Reef in Galveston Bay (Ogle and Flurry 1980), with prevalences ranging from 20 to 80% during the summer and generally less than 10% during the winter and well into the spring. Mean infection intensity was less than 1 (very light to light) during the summer and fell to near-zero in the winter. The simulations using Biloxi Bay conditions (Fig. 14) have many of these characteristics. *P. marinus* prevalence and infection intensity are near zero from December through May. Infection intensity ranges from 2 to 4 on Mackin's Scale, but values above 2 occur for only 2 months (July and August). The Biloxi Bay simulations required a 30 hour halving time rather than the 60 hour halving time used for Galveston Bay to approximate field observations. A higher rate of parasite mortality produced the low *P. marinus* prevalence and infection intensity in the simulated distributions between December and May. The simulated *P. marinus* distributions overshoot observed summer values significantly in only one month, July. Otherwise, values were near or below 1, as observed.

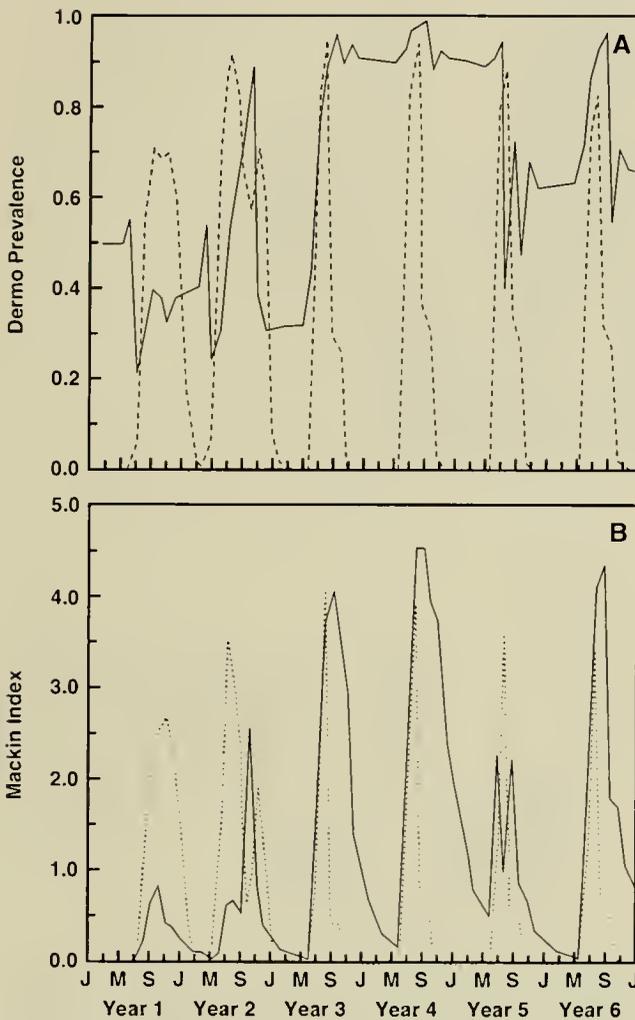


Figure 14. (A) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, from a simulated oyster population that was exposed to reduced salinities typical of Biloxi Bay, MS during the last 2 years of the simulation. Environmental conditions are given in Tables 4 and 5. Prevalence in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (B) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale for the simulated oyster population. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population.

North Inlet, SC had salinities that were higher than those at April Fools Reef in Galveston Bay and temperatures that were similar during the period studied by Crosby and Roberts (1990). This study was based on measurements from relatively large ( $\geq 1$  g tissue wet wt) oysters and included samples taken during an epizootic. *P. marinus* prevalence in the sampled oyster population ranged from 85 to 100% and infection intensity ranged from 1.5 to nearly 4 on Mackin's Scale. For the characteristics of this simulation to match those reported by Crosby and Roberts (1990), parasite mortality rate had to be reduced to a 120 hour halving time and recruitment had to be limited. Crosby and Roberts (1990) do not mention the presence of recruits in the sampled population. Using these conditions, the simulation produces an epizootic in

year 3 which results in sustained adult mortality (Fig. 15). Prevalence in the simulated oyster population approached 100% for most of year 3 (Fig. 15a) and infection intensity exceeded 4 on Mackin's Scale in August through October, falling to 1.0 to 1.5 early in the following spring (Fig. 15b).

The simulations suggest that either parasite division rate or parasite mortality rate must vary regionally among oyster populations. Simulating field observations from different bay systems requires one or both to be modified. Much disagreement exists about relative resistances of oyster populations to *P. marinus* (e.g., Paynter and Bureson 1990, Bureson 1991). The Biloxi Bay and North Inlet simulations suggest that some lability in disease resistance must be present. Elston et al. (1987) pointed out that oyster populations may be resistant to mortality, but susceptible to infection. The simulations described above emphasize the relative resistance to mortality by varying rates of parasite division or parasite mortality. These simulation results are in agreement with experimental results (Bushek and Allen 1994) that show variations in virulence between *P. marinus* populations.

#### Recruitment and Transmission

Parasite transmission is a complex process that is dependent upon many interacting biological and environmental factors (Anderson and Crombie 1984, Burdon et al. 1989, Woolhouse and Chandiwana 1990). Little comparative data on transmission of *P. marinus* is available, even though transmission is a crucial aspect of the disease process. Patterns of settlement and recruitment produce significant variations in prevalence and infection intensity in the population as a whole (cf. Fig. 4a,c). The problem of false negatives compromises the assessment of transmission because transmission and infection intensity cannot presently be separated with certainty. The coupled oyster-*P. marinus* model provides a framework for considering disease transmission in oyster populations.

The results of a simulation in which 20 individuals were allowed to recruit to an uninhabited locale in late May (Julian day 140) are shown in Figure 16. Additional recruitment occurred as these animals spawned in the following years. A typical population of adult oysters develops in about 2 years and this population persists (Fig. 16a). *P. marinus* prevalence and infection intensity in this oyster population reach long-term means about 6 months after the oyster population reaches a steady distribution (Fig. 16b,c). In this simulation, most of the oysters are infected with *P. marinus* about 6 months after settlement (Fig. 16b). A significant mortality event occurred in year 2 of the simulation which resulted in nearly 100% loss of the market-size (2-year old) population (Fig. 16a). The characteristics of this simulation agree with data collected during and after a catastrophic mortality in San Antonio Bay, TX where oysters and *P. marinus* recolonized from an external source, and with various anecdotal and descriptive reports (e.g., Hofstetter 1977, Ray 1987, Andrews 1988, Paynter and Bureson 1991). Thus, the model reproduces field observations of transmission to the extent that comparisons are available.

#### System Memory

Bays and estuaries have occasional years of abnormal environmental conditions. The significance of an aberrant year was examined by replacing year 2 of the April Fools Reef time series, which is conducive to oyster population growth, with one that is deleterious for growth. The remaining 5 years of the time series

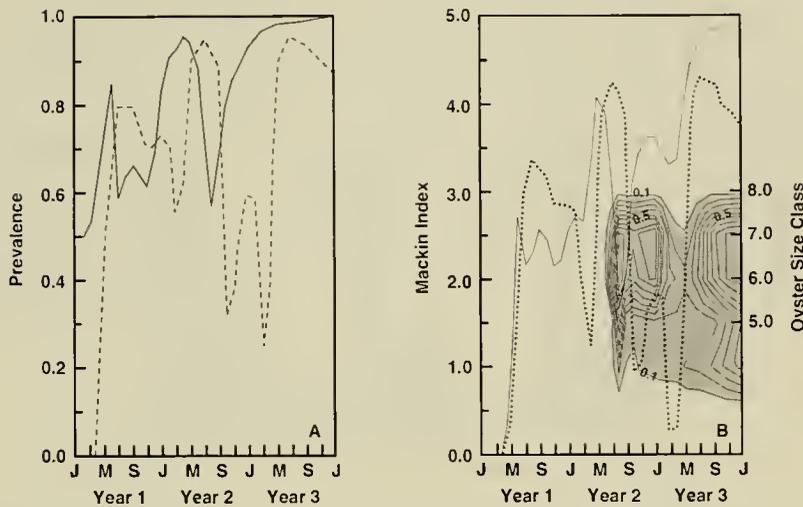


Figure 15. (A) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, from a simulated oyster population that was exposed to high salinity conditions typical of North Inlet, SC in the last year of the simulation. Environmental conditions are given in Tables 4 and 5. Prevalence in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (B) *Perkinsus marinus* infection intensity expressed in terms of Macklin's Scale for the simulated oyster population. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market size ( $\geq 3$  inches) portion of the population. Shaded region indicates population mortality calculated as the fraction of the population in a given size class that die during a one-month period. Contour interval is 0.1.

were unaltered (Table 5). In the first simulation, year 2 was replaced with the conditions measured in North Inlet, SC during an epizootic. The environmental conditions were taken from a drought year and showed salinities that average above 30 ppt. *P. marinus* prevalence and infection intensity in the simulated oyster population did rise transiently in response to the higher salinity (Fig. 17), but no long-term deleterious effect was observed. In fact, the primary effect appears to be a reduction in population mortality (cf. Fig. 15b and 17c). High salinities in year 2 improved reproduction in that year and the number of market-size oysters increased over the long term.

The environmental time series for Dickinson Reef, Galveston Bay is characterized by low food supply and high turbidity. Temperature and salinity at this location are similar to those at April Fools Reef. Four continuous years of the conditions from Dickinson Reef resulted in persistent oyster population declines (cf. Fig. 10a). When the environmental time series from Dickinson Reef is inserted in year 2, the oyster population crashes over a period of 2 years (Fig. 18a). Reproduction in the simulated oyster population ceases 2 years later (Fig. 18b). *P. marinus* infection intensity remains high into the winter (Fig. 18c,d), as does prevalence in year 3. This higher infection level produces the lowered fecundities in the following years that subsequently limit recruitment. Without recruitment of uninfected individuals, an epizootic finally destroys the population (Fig. 18a).

The above simulation illustrates that one extreme year is sufficient to dramatically alter the long-term history of the oyster population. An extreme year, characterized by low food supply, permits *P. marinus* to gradually take control and force the population to extinction. In contrast, an extreme year characterized by high salinity produces little long-term effects in the oyster population. Under certain conditions, a significant system memory exists which allows one year to dictate significant events well into the future. An interesting observation from a census of *P. marinus* in Gulf of Mexico oyster populations (Craig et al. 1989, Wilson et

al. 1990, Powell et al. 1992) is the lack of a correlation between salinity and infection intensity throughout the Gulf of Mexico, although localized correlations exist in nearly every individual bay system. The previous two simulations show that the recent history of environmental change can be an overriding influence on disease prevalence and infection intensity in oyster populations. Sites within a bay will have a similar recent history and, therefore, will retain the correlation between infection intensity and salinity. Sites between bays may have a different recent history, possibly sufficiently different to alter the progression of the disease under similar salinity conditions, and thus will likely not demonstrate a simple regional correlation with salinity. The above simulations further suggest that an extreme year of a climatic cycle could affect the oyster populations for years after climate returns to normal. The importance of climatic cycles in regulating oyster populations in the Gulf of Mexico has recently been noted (Powell et al. 1992, Wilson et al. 1992) and the influence of climatic cycles has been documented for other host-parasite systems (MacKenzie 1987, Rogers and Randolph 1991).

CONCLUSIONS

The coupled oyster population-*P. marinus* model described above suggests a number of characteristics of *P. marinus*-infected oyster populations that have yet to be measured adequately or observed. These include such phenomena as high winter infection intensities, oyster mortality at low salinity due to *P. marinus*, and the importance of lowered transmission rates during the winter. These observations point to the need to more carefully examine the yearly progression of disease under a variety of environmental conditions. The simulated distributions also suggest that the standardly-used assessment of population infection levels can yield inadequate or misleading results because the entire population is not monitored. Clearly, intensive field observations will be required to fully understand the course of infection in oyster populations and the triggering mechanisms for epizootics.

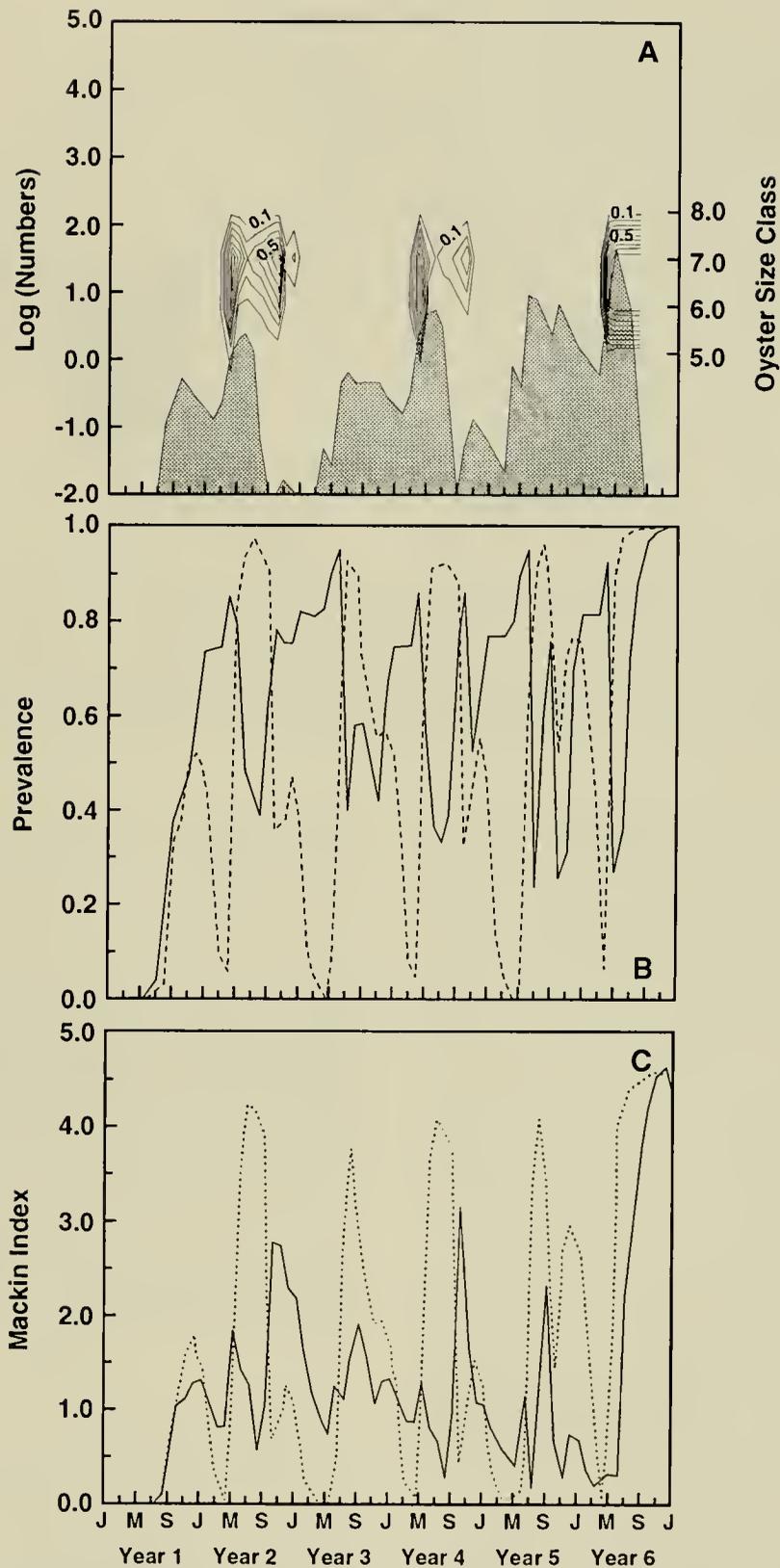


Figure 16. (A) The number of market-size individuals (solid shading), expressed as  $\log_{10}(\text{number m}^{-2})$ , from a simulated oyster population that was initially uninfected by *Perkinsus marinus*. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (B) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (C) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale for the simulated oyster population. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population. Environmental conditions are given in Tables 4 and 5.

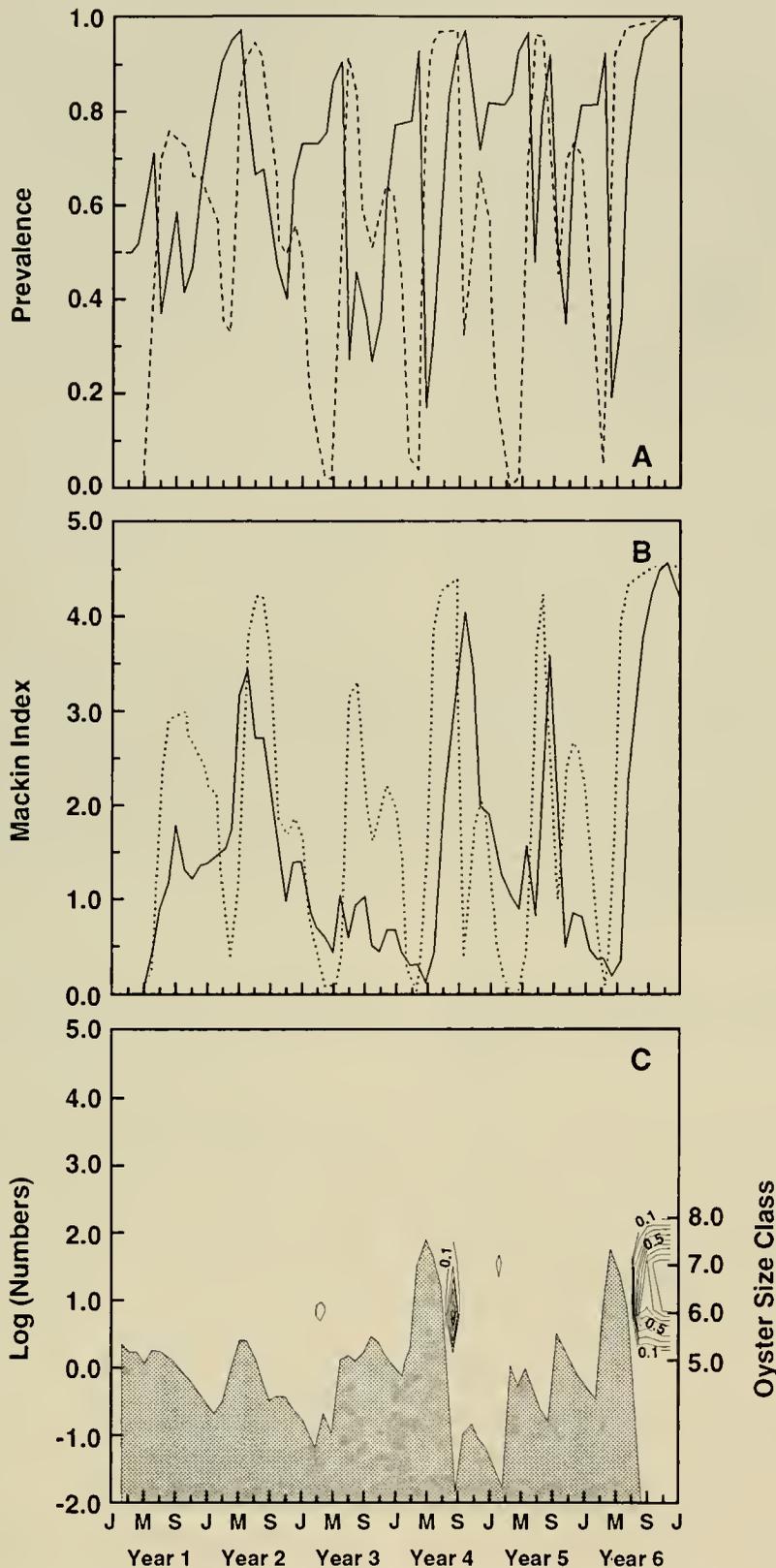
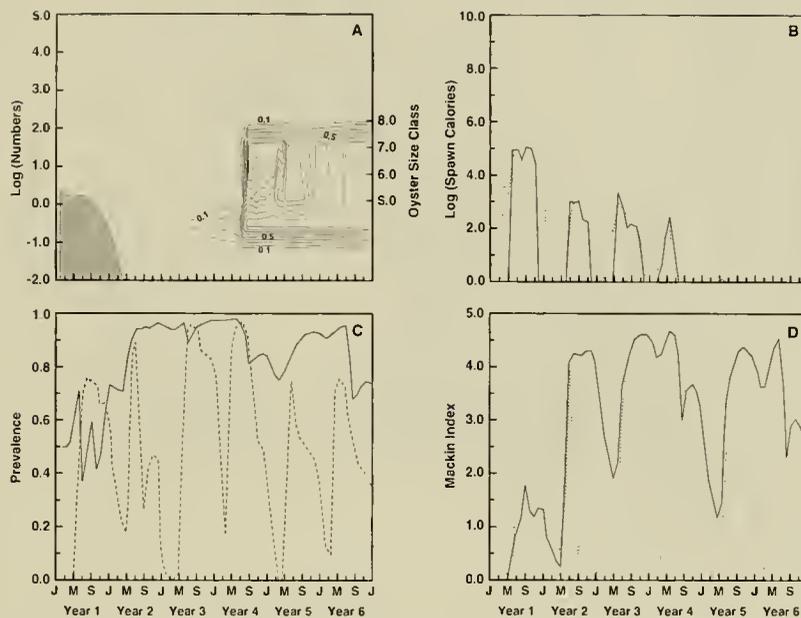


Figure 17. (A) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, from a simulated oyster population that is exposed to high salinity in year two of the simulation. Environmental conditions are given in Tables 4 and 5. Prevalence in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (B) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale for the simulated oyster population. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market size ( $\geq 3$  inches) portion of the population. (C) The number of market-size individuals (solid shading) in the population in terms of  $\log_{10}(\text{number } \text{m}^{-2})$ . Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1.



**Figure 18.** (A) The number of market-size individuals (solid shading), expressed as  $\log_{10}(\text{number m}^{-2})$ , from a simulated oyster population that is exposed to low food and high turbidity conditions in year two of the simulation. Environmental conditions are given in Tables 4 and 5. Mortality events, calculated as the fraction of the population in a given size class that die during a one-month period, are indicated by the contours, with an interval of 0.1. (B) Simulated reproductive effort (solid line) for the oyster population in terms of  $\log_{10}(\text{total Joules spawned per month})$ . The quantity of gametic tissue present in the adult oysters, as  $\log_{10}(\text{total Joules present on day 30 of each month})$ , is shown by the dotted line. (C) *Perkinsus marinus* prevalence, expressed as the fraction of the total population that is infected, in the entire oyster population and the market-size population assuming that all infections  $\leq 2^{12}$  cells  $\text{ind}^{-1}$  are judged negative using the method described in Ray (1966) are represented by the solid and dashed lines, respectively. (D) *Perkinsus marinus* infection intensity expressed in terms of Mackin's Scale for the simulated oyster population. Solid line indicates infection intensity in the entire oyster population; dotted line represents infection intensity in the market-size ( $\geq 3$  inches) portion of the population.

Management of diseased oyster populations must take into account the complexity of the history of disease in oyster populations. The course of infection is not a simple function of temperature and salinity change and is not necessarily predictable from them. Similar temperatures and salinities at various sites around the Gulf of Mexico support oyster populations with different *P. marinus* infection intensities and oyster population densities. Food supply plays an important role since one of the primary defenses the oyster has against the disease is to simply grow faster than the *P. marinus* infection can progress. The evidence suggests that inter-population differences in the competency of the oyster's defense system or in the growth rate of the parasite probably exist.

Most importantly, an extended system memory can exaggerate the importance of extreme years in the history of oyster populations.

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## EVIDENCE FOR DOUBLE RECRUITMENT IN THE COMMON PERIWINKLE, *LITTORINA LITTOREA* (LINNAEUS, 1758), ON PENDLETON ISLAND, NEW BRUNSWICK, CANADA

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**ABSTRACT** A one year study of an intertidal population of *Littorina littorea* situated in a high current passageway on Pendleton Island, New Brunswick, Canada revealed the recruitment of 2 cohorts. Continued observations of the population structure and incorporation of a settlement study confirmed that double recruitment appeared in 2 consecutive years. One probable cause for the observation of double annual recruitment is that of multiple spawning. This hypothesis was investigated through a detailed examination of the breeding cycle. Macroscopic and microscopic examination of the gonads and monthly stereological (gamete volume fraction) values, revealed that the breeding cycle is characterized by a prolonged spawning period rather than multiple spawning. The reason for the double recruitment is still unknown, but data suggest that this may be the result of a temporary cessation in spawning.

**KEY WORDS:** Recruitment, *Littorina littorea*

### INTRODUCTION

The common periwinkle, *Littorina littorea* (L.), is found in the North Atlantic where it inhabits most rocky shores. It is abundant throughout the littoral zone in the Bay of Fundy (Ganong 1889, Newcombe 1935, La Rocque 1953, Bousfield 1960, Thomas 1983), often the most dominant mollusc in this region (Gardner and Thomas 1987). *L. littorea* is therefore of particular importance to the rocky intertidal shore community (Gardner and Thomas 1987).

Although the abundance and wide distribution of *L. littorea* make it an excellent species with which to study reproductive adaptations, there is a paucity of data on variation in the breeding cycle under various environmental situations. Extensive literature exists on the reproduction in individual *L. littorea* (Gegenbauer 1852, Caullery and Pelsener 1910, Linke 1933, Fretter and Graham 1962), however, the majority of the studies on breeding cycle of populations have been conducted in Britain on open coast populations (Tattersall 1920, Elmhirst 1923, Moore 1940, Williams 1964) with the exception of Fish (1972) who compared the breeding cycle of open coast and estuarine populations on the west coast of Wales. In Canada, the breeding cycle has been studied by Hayes (1929) as part of a study on growth. Data on variation in the breeding cycle in different environmental situations is vital in understanding the life history of the organism. This type of information is especially important since *L. littorea* is now being considered a possible bioindicator species (Bakke 1988).

Chase (1989) reported double annual recruitment in a population of *L. littorea* on Pendleton Island, New Brunswick. All previous recruitment studies on *L. littorea* report the addition of one cohort per year (Smith and Newell 1955, Moore 1937, Williams 1964, Fish 1972, Gardner and Thomas 1987). Possible explanation for multiple recruitment include: multiple spawning, differential survival of spat and/or larvae, delay of metamorphosis of larvae, the incursion of larvae or post-settlement individuals from

neighboring populations and differential growth rates of post settling individuals. The purpose of this study was twofold: firstly, confirm the observation of double recruitment through more rigorous population census and the incorporation of a settlement study and secondly, to investigate whether multiple spawning explained the observed recruitment pattern through a detailed analysis of the breeding cycle. In addition to collecting basic qualitative data (Moore 1940, Williams 1964, Fish 1972), quantitative analysis of the reproductive cycle was also performed.

### MATERIALS AND METHODS

#### Study Area

Pendleton Island is located in New Brunswick, Canada at 45°02'N and 67°56'W within the Deer Island Archipelago (Fig. 1). The climate and oceanographic setting, geology and the general ecology have been summarized by Thomas et al. (1990). The collection sites were on a 200 m long section of the shore in Pendleton passage, a sheltered channel with high tidal current (Thomas et al. 1990). Bottom currents reported range up to maxima of 0.47 and 0.60 ms<sup>-1</sup> for ebb and flood of spring tides respectively with a mean velocity of 0.11 ms<sup>-1</sup> (Thomas et al. 1990). The shore is predominantly sedimentary with scattered rock and outcrops. *L. littorea* were abundant.

#### Demography and Settlement Patterns

The population was monitored in 1988 and 1989. Random samples of approximately 1000 *L. littorea*, representing all size classes, were collected from the study site on May 4, July 6 and August 25 of 1988 and May 23, June 20, August 22, September 12 and October 30 of 1989. The spire height (defined as the distance from the spire apex to the furthest tip of the aperture lip) was measured using computer assisted vernier calipers. Gardner (1986) has shown that growth of *L. littorea* cannot accurately be ascertained from thin sections or acetate peels due to the spiral nature of its growth. Therefore, length frequency histograms were plotted and the Harding (1949) method of cohort placement was used to analyze the demographic structure. While it is accepted that cohort identification by such a means may be subjective, despite regular sampling of the populations, we incorporated a settlement study in

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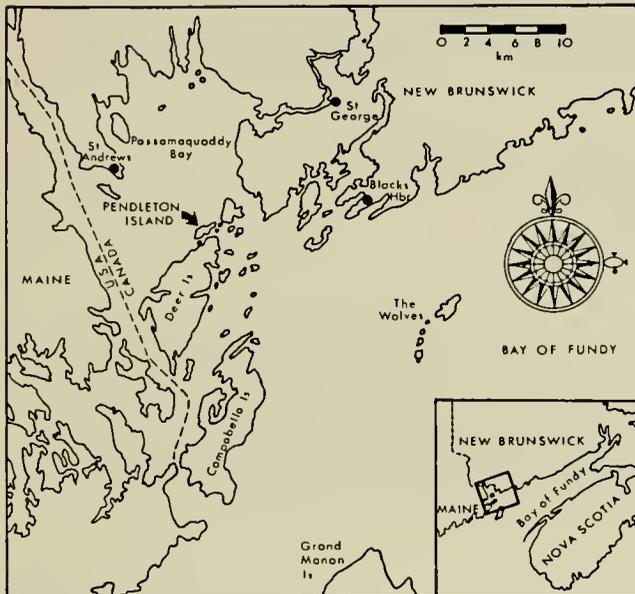


Figure 1. Location of Pendleton Island in the Deer Island Archipelago, New Brunswick.

1989 to confirm the observation of double recruitment in the population. On May 30, 1989 a set of 12 ceramic tiles were placed on the shore 5 cm apart along a transect running perpendicular to the shoreline at the following tidal levels; 0, 20, 40, 60, 80 and 100%. The tiles were monitored every 2 weeks from May 30 to November 6, 1989 for the presence of *L. littorea* spat. Once recruitment began the number and lengths of *L. littorea* were recorded and length frequency histograms plotted. Recruitment was defined by the presence of individuals less than 2 mm. The tiles were to provide evidence for the presence/absence of spat to corroborate the observation of double recruitment, and not a means of estimating the density of recruitment, as we do not feel such an estimate would be a true reflection of density. *L. littorea* is not a stationary mollusc and it was observed that depending upon the time at which the plates were examined (i.e., high tide vs. low tide) the number of *L. littorea* on the plates would vary considerably. Based on the results of regular sampling and the settlement study we are confident of the observation of double recruitment.

#### Sex Ratio and Breeding Cycle

The sex ratio of the breeding population was estimated by collecting monthly samples of approximately 200 sexually mature individuals (spire heights > 13 mm, Williams 1964) from May 1989 to July 1990 each. Chi-square analysis was performed to test for significant departures from the reported sex ratio of 1:1 (Moore 1940, Williams 1964, Fish 1972).

We investigated the breeding cycle using macroscopic and microscopic techniques. Macroscopic examination was employed by dividing the population into stages of development as Williams (1964) and Fish (1972). Both authors recognized 5 stages of *L. littorea*, [i.e., stage I (immature virgin), stage II (maturing individuals/recovering spent), stage III (fully mature and spawning), stage IV (partially spent) and stage V (spent)]. The same stages were used in this study with the exception of stage I (immature virgins) which were not included in this assessment as we were concerned only with the breeding population.

From May 1989 to November 1990, monthly samples of 100 males and 100 females, representing all size classes, were killed by immersion in boiling water for 1 minute. Individuals were then removed from the shell, examined and classified into developmental stages.

In addition to this external examination, microscopic techniques of stereology (Steer 1981) were also employed to assess the different stages of gonad development and to determine the mean gonad volume fraction (GVF) of individuals in each monthly sample. GVF was defined as the proportion of gonadal tissue containing mature and developing gametes. Five males and 5 females of the following spire heights; 15, 20 and 25 mm were removed from their shells after relaxing them in methanol, fixed in Bouin's solution and stored in 70% ethanol. Tissues were prepared for sectioning and microscopic examination using standard methods. All snails were classified by stage of gonad development and the number in each stage was recorded for each sex.

## RESULTS

#### Demography and Settlement Pattern

Figure 2 shows length-frequency histograms of the population of *L. littorea* on Pendleton Island for May, July and August 1988 (Fig. 2 a, b, c) and May, June, August, September and October 1989 (Fig. 2 d, e, f, g, h) to illustrate the double recruitment pattern observed for this population in the 2 years. More detailed analysis of the population structure can be found in a companion paper (Chase 1991). In 1988 the first recruitment phase was in July with the  $0^+$  cohort which had a mean spire height of 3.7 mm and comprised 9.3% of the population. The second phase of recruitment was in August, the  $0^{++}$  cohort, which had a mean spire height of 2.5 mm and comprised 13.3% of the population. By the second phase of recruitment, the  $0^+$  cohort had already attained a mean spire height of 6.8 mm. In 1989, the first phase of recruitment was in August with the  $0^+$  cohort which had a mean spire height of 4.9 mm and comprised 21.8% of the population. The second phase was first detected in October, the  $0^{++}$  cohort, which had a mean spire height of 2.5 mm and comprised 30% of the population. By the second phase of recruitment, the  $0^+$  cohort had already attained a mean spire height of 7.3 mm. The differential timing of the recruitment of the new cohorts ( $0^+$  and  $0^{++}$ ), July and August 1988 and August and October 1989, was the result of local temperature variation (Chase and Thomas in press).

Figure 3 shows length-frequency histograms of combined data from the 12 tiles located at high (80%) tidal level. No recruitment of *L. littorea* was observed at the 100, 60, 40, 20 or 0% tidal levels. The first phase of recruitment was observed on August 10, 1989. Eight individuals were observed on the tiles with mean spire height of 1.08 mm ( $\pm 0.23$ ). The second phase of recruitment was first observed on September 7 [n = 49, mean spire 0.99 mm ( $\pm 0.59$ )], but was very prominent in the September 20 sample [n = 253, mean spire height 0.89 m ( $\pm 0.02$ )].

#### Sex Ratio and Breeding Cycle

The proportion of female to male *L. littorea* was slightly higher in samples taken from May 1989 to July 1990 than for the remainder of the year. However, with the exception of September 1989, the sex ratio did not differ significantly from a ratio of 1:1.

The breeding cycles of female (Fig. 4a) and male (Fig. 4b) *L. littorea* at Pendleton Island are represented in diagrammatic form

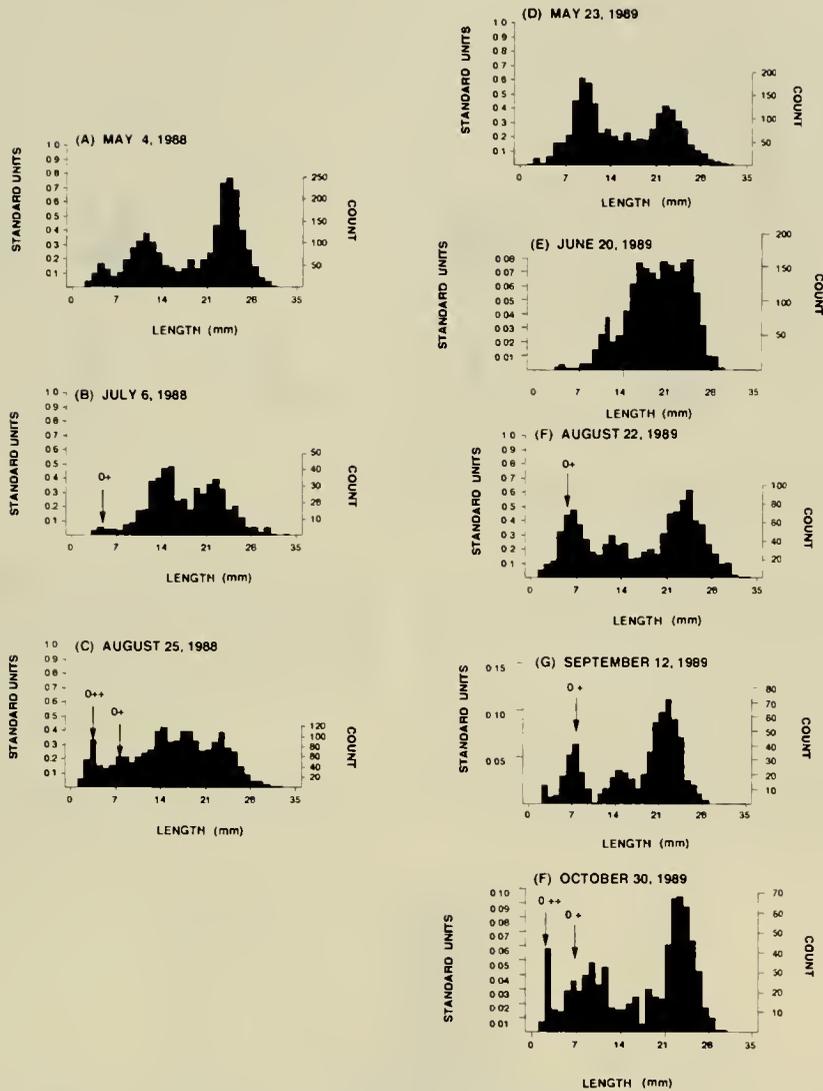


Figure 2. Length-frequency histograms of the *Littorina littorea* population at Pendleton Island. (A) May, (B) July, (C) August 1988, (D) May, (E) June, (F) August, (G) September, (H) October 1989. Arrows shows new recruitment.

for the 1989 and 1990 seasons; the relative proportion of specimens in each developmental stage at each collection is expressed as a percentage for each month of the year. Males began to mature before females. In January and February 100% of the females were still at stage II (maturing), whereas, by February 29% of the males had already reached full maturity (stage III). Females matured rapidly from 0% mature in February to 51 and 56% mature in March and April, respectively. Copulation appears to have begun in mid June in 1989 coincidental with the increase in the percentage of males in stage IV (partially spent), 63% in July compared to 0% in May and 10% June. Copulation appears to have begun earlier in 1990, late April or early May [percentage of males in stage IV (partially spent), 23% in May compared to 0% in April]. In addition, spermatozoa were evident within the bursa copulatrix and receptaculum seminis of the females in June (1989) and May (1990). In both years there was no indication of multiple spawning, the decrease in stage II (developing) individuals supports this conclusion. No redevelopment was apparent in the gonadal tissue until after the population was spent. In both 1989 and 1990 the increase in the number of stage V (spent) females was initially

slow. In 1989 the percentage of females in stage V was only 50% in September but increased rapidly from September (50%) to October (100%). In 1990 the number of stage V females was only 48% in September but increased rapidly to 81% in October and 100% in early November. The entire population was spent (stage V) by late October/early November of each year.

**Gamete Volume Fraction (GVF)**

Seasonal cycles in volume fraction for the male and female gonads were determined (Fig. 5) for the population at Pendleton Island in addition to the stages described above. Maximum values for males and females, indicating full maturity, occurred in June 1989 (males 67, females 38%) and in May 1990 (males 79, females 42%). Copulation occurred later in 1989 (mid-June) than in 1990 (early May), as indicated by the decrease in GVF for males in each of those months and the presence of spermatozoa in the bursa copulatrix and receptaculum seminis of the females. Female GVF decreased only marginally after copulation was known to have occurred (1989: 38% June, 30% August; 1990: 43% May,

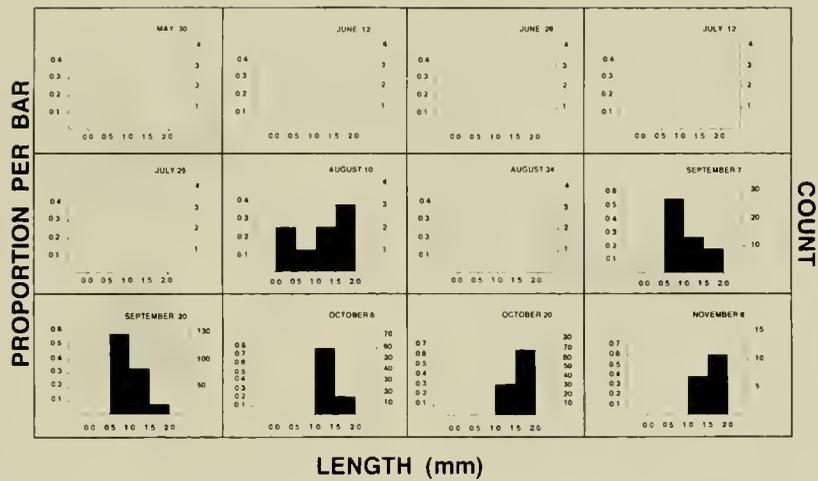


Figure 3. Length-frequency histograms of *Littorina littorea* observed on plates at high (80%) tidal level.

40% June, 41% July and 41% August). No large decrease in female GVF was observed until October 1989 (30% August to 12% October) and September/October 1990 (41% August to 22% in September and 13% in October). Minimum values of approximately 12.0% for the females and 15.0% for the males for total gamete volume were observed in October/November in both 1989 and 1990, just after spawning. Increases in the gamete volume fraction between December and January are largely the result of an increase in the percentage of developing gametes in the gonadal

tissue in both the males and females during a period when fully developed gametes were not yet present. The maximum GVF values were proportionately higher in males than the females in both 1989 (67 vs. 38%) and 1990 (79 vs. 42%).

DISCUSSION

Results indicate that the spawning period of *L. littorea* on Pendleton Island extends from mid June to September or October.

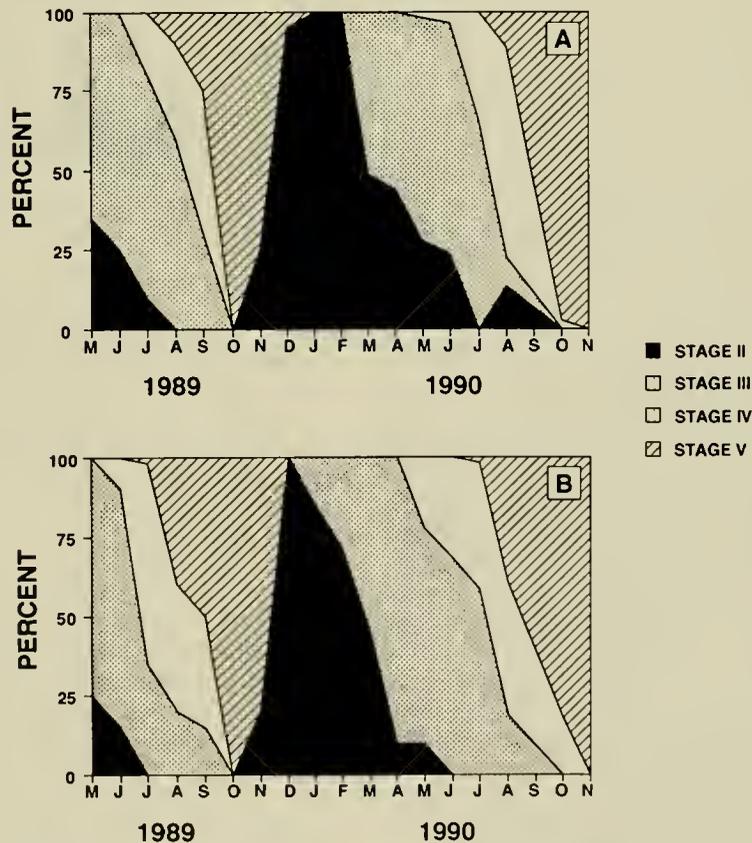


Figure 4. Breeding cycle of *Littorina littorea* at Pendleton Island. (A) females, (B) males, May 1989 to November 1990.

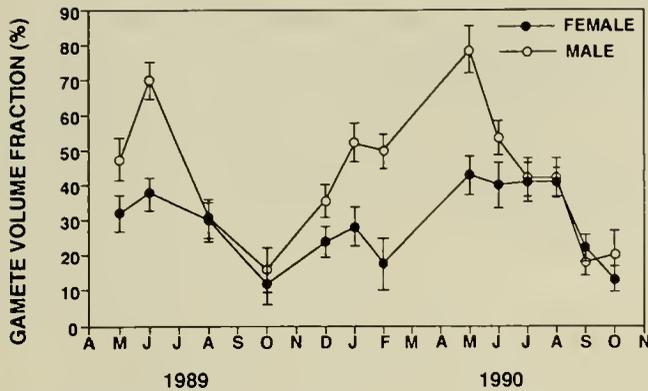


Figure 5. Gamete Volume Fraction for *Littorina littorea* on Pendleton Island, May 1989 to October 1990.

The gradual decrease in the GVF (1989 and 1990) and the percentage of the population in the stages IV (partially spent) and V (spent), from June to October (1989) and from May to November (1990) support this conclusion. There was no evidence from histological examinations that the population underwent a second period of gametogenesis, thus ruling out multiple spawning. Therefore, the observed settlement in 1988 and 1989 is the result of a single prolonged spawning incident.

The reason for the presence of 2 distinct cohorts per year, however, is less certain. Of the many hypotheses (e.g., delay in larval metamorphosis, incursion of larvae from neighboring populations) that may explain this phenomenon, the one most strongly supported by our results is a temporary cessation and subsequent continuation of spawning. In both 1989 and 1990 there was a

partial decrease in female GVF (initial spawning) after copulation was known to have occurred (Fig. 5). The GVF then either decreased fractionally (1989) or remained constant (1990) before decreasing rapidly (second spawning) to the minimum, spent condition. Initial spawning resulted in low recruitment density on the shore, consisting of individuals capable of rapid growth (1–2 mm per month, Hayes 1927). In this study the first phase of recruits had attained a spire height of approximately 7 mm in both years before the next phase of recruits appeared on the shore (Fig. 2). This rapid growth may be a reflection of the high productivity of this sheltered channel (Thomas et al 1990). The size differential was maintained since the second phase of recruits were subject to similar environmental factors that affected the growth of the first settlers. These 2 groups appeared as distinct cohorts in length frequency histograms (Fig. 2). Future research should concentrate on obtaining regular plankton samples as well as analysis of reproductive cycles of neighboring populations to confirm our results. It would be of interest to conduct genetic analyses of the populations of *L. littorea* at different locations within the passage-way. It is possible that the population may be extremely heterozygous and any variation in the reproductive patterns may be a reflection of variation at the genetic level.

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## SEXUAL TRIMORPHISM IN *STROMBUS LUHUANUS* LINNÉ, 1758 (MOLLUSCA: GASTROPODA) AT SHIRAHAMA, JAPAN

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**ABSTRACT** *Strombus luhuanus* collected at Shirahama, Japan showed sexual trimorphism. Masculinized females were larger for all linear and weight measurements than either males or normal females. Such females also produced more tissue to shell, indicating a greater advantage for mariculture if this characteristic can be exploited. Normal females were not larger than males as has been reported in other species of the genus *Strombus*, but were found to differ slightly in shell shape. Both types of females had slightly more slender shells than males as indicated by width/length ratios.

**KEY WORDS:** *Strombus*, trimorphism, masculinization

### INTRODUCTION

Sexual dimorphism is characteristic of the Strombidae, with males possessing a penis (verge) and females, an egg groove. Size differences between the 2 sexes is also apparent for many of the strombid species studied so far with females possessing a larger shell than males (Colton 1905, Goodrich 1944, Abbott 1949, 1960, Robertson 1959, Randall 1964, Alcolado 1976, Blakesley 1977, Kuwamura et al. 1983). None of these studies, however, had looked at whether size differences extended to the animals themselves until Reed (1993b) showed that female *Strombus pugilis* were heavier on average than males when tissue weight was considered; shells were also heavier due to their larger size.

Differences in size were also found in a third morph, masculinized female *S. pugilis* (Reed 1993b). Masculinized females were found to be larger than normal females which were in turn larger than males. This difference in size was due to growth to an overall larger size, not to differences in shape. Although masculinized females have been reported for other species of strombids, a paucity of specimens did not allow in depth comparisons (Reed 1992). Kuwamura et al. (1983) first described this condition in *Strombus luhuanus*, but considered masculinization normal as 71% of females were affected; consequently, those authors did not look at differences in size among the 3 classes, only between males and females in general.

This study was undertaken to characterize size differences among the 3 morphs in *Strombus luhuanus*. Detailed descriptions of the masculinized condition, in which females possess both an egg groove and a small, often deformed verge, may be found in Kuwamura et al. (1983) and Reed (1992 or 1994, 1993a).

### MATERIALS AND METHODS

The data for *S. luhuanus* were provided by Dr. T. Kuwamura from the original studies done on that species (Kuwamura et al. 1983, Wada et al. 1983) at Shirahama, Japan. Approximately 40 animals were collected monthly from June 1976 to June 1977. Measurements taken were LENGTH, length of the shell from the siphonal canal to the tip of the spire (mm), WIDTH, width of the body whorl (mm), TOTAL wt., total weight of live animal and shell (g), ANIMAL wt., weight of animal after removal from the shell (g), and GONAD wt., weight of the gonad. SHELL wt., weight of the shell only (g), was determined by subtraction of ANIMAL wt. from TOTAL wt. Other data recorded were length

and shape of the verge for all animals. A total of 624 animals were collected: 292 males, 93 normal females, and 239 masculinized females. Lip thickness was not recorded for the specimens; consequently, all specimens are assumed to be of the same age class and thus, of representing one discrete population.

Three ratios were generated from the data in order to test for differences in shape of the shell and tissue allocation among the 3 morphs. The ratio, WIDTH/LENGTH, was used to examine shell shape, and ANIMAL/SHELL wt. was used to test for differences in somatic tissue production versus shell deposition, while GONAD/ANIMAL wt. was used to determine if differences existed in the allocation of tissue resources.

Analysis of variance was used to test for differences among the 3 morphs: masculinized females, and normal females, and normal males. Scheffe multiple comparison tests were used to distinguish which morphs differed. For all statistical analyses, the probability level chosen was 0.05.

### RESULTS

Table 1 presents the means and standard deviations for males, normal females, and masculinized females of *S. luhuanus*. No significant differences were found between males and normal females for linear or weight measurements, or for the 2 weight ratios. Males did show a significantly larger WIDTH/LENGTH ratio than either normal or masculinized females, indicating that the male shell is slightly squatter than the female shell.

Masculinized females were significantly larger than either males or normal females for both size and weight characters. The ratio of ANIMAL/SHELL weight was also significantly greater, indicating that masculinized females produce more tissue than shell relative to the other 2 morphs. The ratio of GONAD to ANIMAL weight was the same for all of the morphs; thus, there is no differential production of gonad tissue among the 3 types.

### DISCUSSION

There is sexual dimorphism between males and females of *S. luhuanus* if all females are considered equal as originally reported by Kuwamura et al. (1983). However, if females are divided into normal and masculinized types and then compared to males, sexual trimorphism becomes apparent.

Males and normal females did not differ in size, but did show a slight variation in shell shape that indicates females have a more

TABLE 1.

Means and standard deviations for the *Strombus luhuanus* data from Shirahama, Japan (prob. level: 0.05).

Measurement	Males (n)	Normal Females (n)	Masculinized Females (n)
LENGTH (mm)	48.1 ± 3.2 (292)	48.0 ± 2.7 (93)	50.3 ± 2.9 (239) <sup>a</sup>
WIDTH (mm)	26.9 ± 1.8 (292)	26.5 ± 1.7 (93)	27.8 ± 1.7 (239) <sup>a</sup>
TOTAL wt. (g)	17.5 ± 2.9 (275)	17.9 ± 2.8 (89)	19.5 ± 3.1 (227) <sup>a</sup>
ANIMAL wt. (g)	4.16 ± 0.78 (292)	4.26 ± 0.77 (93)	4.98 ± 0.87 (239) <sup>a</sup>
SHELL wt. (g)	13.3 ± 2.3 (275)	13.6 ± 2.3 (89)	14.5 ± 2.4 (227) <sup>a</sup>
GONAD wt. (g)	0.14 ± 0.06 (292)	0.15 ± 0.07 (92)	0.18 ± 0.09 (239) <sup>a</sup>
WIDTH/LENGTH	0.56 ± 0.03 (292) <sup>b</sup>	0.55 ± 0.03 (92)	0.55 ± 0.03 (239)
ANIMAL/SHELL	0.31 ± 0.05 (275)	0.32 ± 0.05 (89)	0.35 ± 0.05 (227) <sup>a</sup>
GONAD/ANIMAL	0.03 ± 0.01 (292)	0.04 ± 0.01 (92)	0.04 ± 0.02 (239)

<sup>a</sup> Significant difference between masculinized females and both males and normal females.<sup>b</sup> Significant difference between males and females of either type.

slender shell than males. Masculinized females also differed significantly from males in the WIDTH/LENGTH ratio. Differences in shell shape were not found between the sexes in *S. pugilis* (Reed 1993b) and have not been reported for any other species of the genus as yet.

Masculinized females were significantly larger than either males or normal females. The ratio of ANIMAL to SHELL weight was also larger, indicating that masculinized females produced more tissue than shell relative to the other 2 morphs (this difference was not accounted for by variation in gonad production). This finding was not seen in *S. pugilis*, although the available sample

size for weights of masculinized females used in that analysis was low (n = 7, Reed 1993b). If, however, this ratio is a true reflection of differential tissue production, then the exploitation of masculinized females for the purposes of mariculture becomes even more important than originally hypothesized (Reed 1994).

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## INDUCED TRIPLOIDY IN THE CHILEAN BLUE MUSSEL, *MYTILUS CHILENSIS* (HUPE, 1854), AND PERFORMANCE OF TRIPLOID LARVAE

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**ABSTRACT** Triploidy in embryos of the Chilean blue mussel, *Mytilus chilensis*, was induced with heat shock using 32°C applied for 10 min, 10 and 40 min post insemination (blocking meiosis I and meiosis II respectively) in zygotes incubated at 18°C. Four separate experiments in time were carried out using different stocks of progenitors. Ploidy levels were assessed by chromosome counting in embryos at 20 hrs. The diploid number (2N) for *M. chilensis* was found to be 28 and therefore triploids (3N) presented 42 pairs of chromosomes. The highest percent of triploids was obtained in embryos treated 10 min post egg activation (51%) in experiment 4. Low percentages of triploids were obtained (ranging 15 to 51%), in relation with results using heat shock reported in the literature. Low survival in the cultures was observed, caused mainly by the high proportion of aneuploids that did not reach the D-stage larvae. We observed a significant difference for growth in shell length between controls and treated larvae after 15 days of culture.

**KEY WORDS:** *Mytilus chilensis*, triploidy, genetics, Chile

### INTRODUCTION

The Chilean blue mussel *Mytilus chilensis* (Hupe 1854) is an economically important bivalve in southern Chile. Its culture began in 1943 in the area of Quellón located southeast of Chiloé Island (Navarro and Gutierrez 1990) and the aquaculture production for this species in 1993 was 3,864 t (Aiken 1993). The Chilean blue mussel was chosen as the test species for inducing triploidy since heat shock has been tested on several species of mussels, although chromosome number in *M. chilensis* is unknown.

Triploid organisms contain 3 genomes instead of the usual 2. Chemical pressure and thermal treatments have been reported to induce triploidy in bivalves (Beaumont and Fairbrother 1991). To induce triploidy in bivalves, heat shock treatments at meiosis I or II are advantageous because of the low cost, the safe handling, and the large amount of eggs that can be treated (Thorgaard 1986, Gosling and Nolan 1989). Among the species of mussels that have been induced by heat shock treatment to produce triploids are *Mytilus edulis* (Yamamoto and Sugawara 1988, Beaumont and Kelly 1989, Yamamoto et al. 1990), *Mytilus galloprovincialis* (Scarpa et al. 1994). In this paper we present the results from preliminary experiments on the induction of triploid embryos in *M. chilensis* by applying heat shocks at 2 different times after insemination, and determine the survival and growth between the treated larvae.

### MATERIALS AND METHODS

#### *Mussels and Gametes*

Mature adult individuals of *M. chilensis* (N = 150) from a mussel farm located in Yaldad Bay (43°08'S, 73°44'W) were induced to spawn in the Quempillén laboratory, Chiloé Island, southern Chile. The mussels, after about 4–5 hrs out of water, were rinsed in clean seawater and placed in a 20 L plastic tray with filtered (1- $\mu$ m) and U.V. treated seawater (FSW) at 18°C and were continuously monitored to change them to individual beakers once they start spawning. The eggs and sperm were held about 1 hr at

room temperature (18°C) before use, to help synchronize the egg stage (Scarpa and Allen 1992). Four experiments were carried out during spring–summer 1993, using different stocks of mussels. In each experiment the insemination (pooling eggs and sperm from several progenitors) was done at 18°C, and 10 min after sperm addition, the eggs were rinsed and resuspended in 2 L FSW.

#### *Triploid Induction*

Approximately 5.2 million activated eggs were distributed in six 300 mL baskets with 20  $\mu$ m mesh screen in the bottom. These plastic baskets were maintained at 18°C prior the treatments. After 10 and 40 min post egg-activation a heat shock (32°C) of 10 min of duration was applied, resulting in 2 treatments with 2 controls held at 18°C in similar plastic baskets. After the treatments the activated eggs were rinsed and placed in 10 L buckets at 18°C. The choice of beginning treatments at 10 and 40 min after sperm addition to suppress polar body I and II respectively, was based on previous cytological data, where polar body I was observed at 15 min at 18°C, and polar body II at 35 min (Sastre 1994). After 20 hrs a sample of embryos was placed in Colchicine 0.05% for 60 min, followed by a 15 min hypotonic shock 3:1 (seawater:distilled water) and Carnoy 3:1 (methanol:glacial acetic acid) fixation changed 3 times and stored at 4°C (Beaumont and Kelly 1989). Survival to D-stage was determined by taking counts of D-stage larvae in 5-mL aliquots 24, 48 and 72 hrs later using Uthermöhler cameras observed under an inverted microscope Zeiss IM35.

#### *Ploidy Examination*

The removal of the vitellus was done following Von Brand et al. (1990). To estimate percent triploidy, metaphase spreads were prepared using the "dropped" technique (Quillet and Panelay 1986), chromosomes were stained with hot (45°C) 2% acetic-orcein and metaphases from 40 embryos in each treatment ( $2N = 28 \pm 2$  and  $3N = 42 \pm 2$ ) were examined. The percentage of cells for each ploidy level was estimated from the generated frequency distribution (Wada et al 1989) and used as the value for the larval population.

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### Growth and Survival

In all experiments the embryos from each treatment were placed in 10 L plastic buckets containing FSW at 18°C, at a density of 100 individuals per mL. After 24 hrs (D larvae stage) the density was adjusted to 5 larvae per mL. Every 3 days the larvae were filtered, counted to estimate survival and 3 random samples of 30 larvae from each bucket measured along the longest shell axis until the larvae were 15 days old, close to the pediveliger stage. All the larvae cultures were fed *Isochrysis galbana* T. at a ration of 60–80 cell/ $\mu$ L (Newkirk et al. 1977).

### Data Analysis

The effect of treatments "time post-activation" on survival and triploidy induction was tested using single factor analysis of variance (ANOVA) after arcsin transformation, using the SYSTAT 5.1 (Wilkinson 1991). Statistical analysis was performed on growth data using single factor ANOVA.

## RESULTS

### Triploidy Induction

The diploid chromosome number was determined as 28, on the basis of 285 metaphase spreads (Fig. 1). The percentage of diploid, triploid and aneuploid embryos at 20 hr post egg-activation and larvae survival at day 15 in each treatment for each experiment are shown in Table 1. No triploid embryos were detected in the controls; however, a small percentage of aneuploids were detected in experiment 1 and 3. The triploidy induction in the experiments ranged from 15% to 51%. A higher efficiency of induction can be observed when applying the heat shock at 10 min after sperm addition in the last 3 experiments (Table 1); however, over all experiments no significant effect was found for the variable "time after insemination" (TAI) at 10 and 40 min, on the percentage of triploids ( $F_{3,1} = 0.385$ ,  $p = 0.57$ ). A significant effect ( $t$ -test:  $p < 0.05$ ) of the TAI on the percentage of triploids was found in experiment 4 [targeting meiosis I (10 min) was more efficient in triploid production]. A high proportion of aneuploids were observed in treated larvae in the experiments (Table 1).

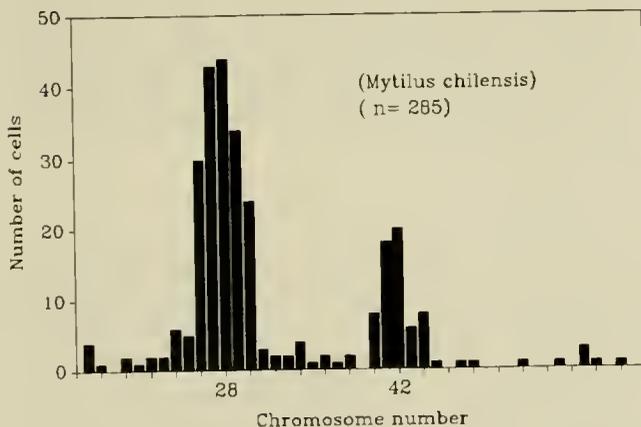


Figure 1. Histogram generated from chromosome number in 20 hr heat-shock treated embryo cells from *Mytilus chilensis*.

TABLE 1.

Percentages of diploids (2N), triploids (3N), aneuploids (XN) in 20-hr-old embryos for each experiment and percentage of survival for each experiment (% SURVIVAL).

Experiment	TAI	2n %	3n %	Xn %	Survival %
1	10	68	24	8	25
	40	55	31	41	17
	Control	98	2	66	
2	10	59	32	9	23
	40	63	26	11	15
	Control	100		77	
3	10	69	25	6	32
	40	74	15	11	14
	Control	97		3	68
4	10	43	51	6	29
	40	60	31	9	13
	Control	100		79	

% SURVIVAL estimated after 15 days of culture at 18°C in the controls and in the treated larvae at different times (min) and after insemination (TAI).

### Larval Development

The presence of a small amount of asynchrony in the larval development was detected. The proportion of D-stage larvae at 24, 48 and 72 hrs was reduced by the treatments (Fig. 2). In 10 min treatments the proportion of D-stage larvae was higher than for the 40 min heat shocks. After 72 hrs of culture the proportion of D-stage in both treatments was higher (Fig. 2) most probably as a result of abnormal and aneuploid larvae decreasing in number. The percentages of larvae surviving after 15 days of culture for both treatments and all experiments are reported in Table 1. A significant effect of TAI was detected on the percentage of survival at 15 days of larvae culture ( $F_{3,1} = 15.7$ ,  $p = 0.03$ ). The percentages of survival obtained for the larvae in the treatments were lower and vary significantly from the controls ( $F_{3,1} = 35.6$ ,  $p = 0.87$ ). No significant differences for growth in shell length between the experiments were detected ( $F_{3,1195} = 0.222$ ,  $p = 0.74$ ); nor for the effect of TAI ( $F_{3,1} = 0.28$ ,  $p = 0.87$ ). Daily growth rates aver-

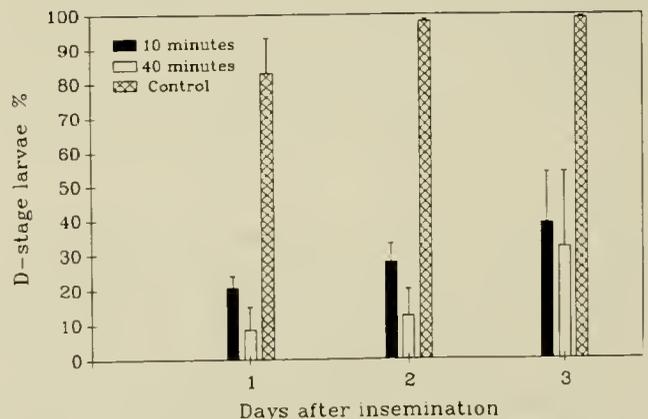


Figure 2. Percentage of D-stage in the treated individuals and in the controls at 24, 48 and 72 hrs after egg-activation in cultures at 18°C. Vertical bars represent standard error.

aged  $4.3 \mu\text{m day}^{-1}$  in the treated larvae and  $6.6 \mu\text{m day}^{-1}$  in the control groups. The mean shell length of the control groups was greater than that treated larvae (Fig. 3) ( $F_{1,2395} = 1085.7$ ,  $p = 0.001$ ).

### DISCUSSION

Diploid individuals of *M. chilensis* had a chromosome complement of  $2N = 28$  while triploids had  $3N = 42$  (Fig. 1). MacDonald et al. (1991) and Koehn (1991) have considered (based on 8 enzyme loci) *M. chilensis* synonymous with *M. edulis*. According to Sarver and Foltz (1993), any debate about species level taxonomy is largely dependent on the species definition which is used. However, and in order to clarify this dispute about genetics taxonomy of species in the genus *Mytilus*, additional enzyme loci, from other characters such as mitochondrial DNA, and from additional locations, will be needed.

Studies carried out in other species of mussels e.g., *M. edulis*, *Mytilus californianus* (Amhed and Sparks 1970), *M. galloprovincialis* (Thiriou-Quévieux 1982), have shown also that the diploid number is constant in the Genus *Mytilus* and corresponds to  $2N = 28$ . In other species of the Family Mytilidae present in southern Chile such as *Aulacomya ater* with  $2N = 26$  (Thiriou-Quévieux 1984), *Choromytilus chorus* with  $2N = 30$  (Palma-Rojas et al. 1980) and *Perumytilus purpuratus* with  $2N = 34$  (Alvarez-Sarret 1991) the number of chromosomes are not conservative among the Genus. The chromosomal information on *M. chilensis* described here should be useful in monitoring triploidization and other kinds of chromosome-set manipulation in this species.

The results of triploid induction obtained from this preliminary study show that all the treatments were effective in producing triploid embryos in *M. chilensis*, reconfirming the effectiveness of heat shock to induce triploidy in bivalve molluscs (Scarpa et al. 1994). Heat shock treatment has the advantage that it is a low cost method that can be easily applied to commercial operations and does not require the specialized skills necessary for the safe handling and disposal of toxic chemicals such as cytochalasin B (Gosling and Nolan 1989). The optimal procedure for triploidy induction and percentage of survival at 15 days of culture was the heat shock ( $32^\circ\text{C}$  for 10 min) applied 10 min after insemination (meiosis I). These results are in accordance with the literature in which the heat shock tends to produce higher percentages of triploids

when blocking meiosis I instead of meiosis II (Quillet and Panelay 1986, Yamamoto and Sugawara 1988, Yamamoto et al. 1988, Gosling and Nolan 1989, Canello et al. 1992).

The percentages of triploidy (24–51%) obtained by blocking meiosis I in the present study are comparable with those reported for other bivalve species, *Crassostrea gigas* (25–45%, Quillet and Panelay 1986), *Tapes semidecussatus* (23.1–55.6%, Gosling and Nolan 1989), *M. edulis* (25–67%, Beaumont and Kelly 1989) but less effective with those reported for *M. edulis* (97.4% using a wide range for  $3N$  number of chromosomes (35 to 48, Yamamoto and Sugawara 1988) and *M. galloprovincialis* (78–84%, Scarpa et al. 1994). However, the heat shock is less effective compared with chemical (CB) treatment in other species of bivalves with percentages of triploids ranging 66–94% in *Argopecten irradians* (Tabarini 1984), over 96% in *Crassostrea virginica* (Barber et al. 1992), 88.2% in *Chlamys nobilis* (Komaru et al. 1988); 78.5% in *Chlamys varia* (Baron et al. 1989), 84–88% in *M. galloprovincialis* (Scarpa et al. 1994). According to Allen (1987), one reason for these differences in ploidy production is that heat shocks inhibit all development, and only those eggs that were in a specific stage of cell division will be affected by the high temperature, while CB does not appear to arrest development of eggs so that zygotes reach the vulnerable stage of cell division during treatment by the chemical.

In the present study the proportion of D-stage larvae were reduced in both treatments compared to the control, an observation that is accordance with other reports in the literature (Canello et al. 1992, Scarpa et al. 1994). The higher percentage of D-stage larvae was found in the 10 min TAI treatment at 24, 48 and 72 hrs and this can be related to the discussion that follows. The induction of aneuploid in the treated larvae of *M. chilensis* might be a cause for the drastic reduction in survival in the cultures, especially those treated at 40 min post insemination [aneuploidy appears to be very common in *M. edulis*, particularly in stressful environments (Dixon 1982)]. But the higher survival obtained in the treated groups by blocking meiosis I (Table 1) might not be completely a result of aneuploid larvae per se but rather of the genetic composition of triploids produced when first meiosis division is blocked (Beaumont and Kelly 1989). The greater heterozygosity resulting from the heat shock application at 10 min (Stanley et al. 1984) could be the cause of a better survival in these groups of larvae (Zouros et al. 1983, Mallet et al. 1985, Diehl and Koehn 1985). Also Singh and Zouros (1981) suggested that greater heterozygosity in *C. virginica* would increase performance in aquaculture.

The lack of effect of TAI on percent of triploidy in 3 out of 4 experiments in the present study, might be a cause of some asynchrony in the larval development, partially due to a premature activation of some eggs during the spawning.

Larval growth rates in triploidy experiments have been carried out by Downing and Allen (1987) in *C. gigas* and by Baron et al. (1989) in *C. varia*; these authors, in contrast with the present study in *M. chilensis*, observed no significant difference for growth in length between control and treated larvae. In contrast with the results of the present study, Beaumont and Kelly (1989) reported that meiosis I induced triploids showed a mean shell length of 36-day-old larvae that were significantly higher than the means of control groups and triploids produced by blocking meiosis II.

In the present study we estimated that percent of ploidy production from chromosome counting of randomly examined metaphase of 20-hr-old embryos. Further studies are needed to examine the ploidy of the spats to verify these results because some authors

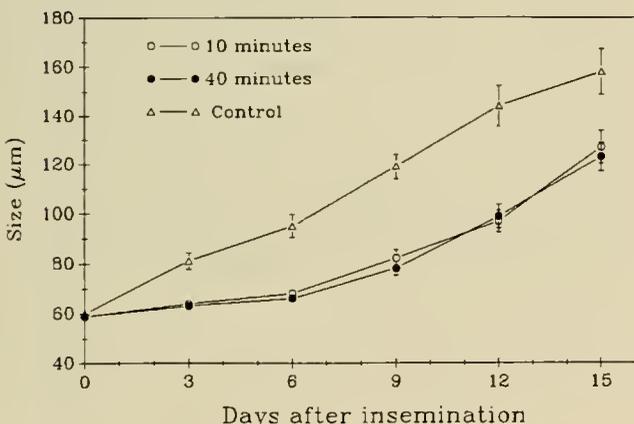


Figure 3. Growth curves of controls and treated ( $32^\circ\text{C}$ , 10 min of duration at 10 and 40 min post insemination) larval population. (*M. chilensis*) cultured under  $18^\circ\text{C}$  over 15 days. Pooled data from all experiments. Vertical bars correspond to confidence intervals.

have reported that percent of polyploid larvae sometimes is reduced during larval stage in other bivalve species (Uchimura et al. 1989). Also, further studies, including a combination of heat and caffeine (Yamamoto et al. 1990) to induce triploidy in *M. chilensis* will be carried out in this laboratory.

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## REPRODUCTIVE CYCLE OF THE CHILEAN RIBBED MUSSEL *AULACOMYA ATER* (MOLINA, 1782)

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**ABSTRACT** Individuals of *Aulacomya ater* (Molina 1782) under suspended culture at Yaldad Bay showed a continuous gamete release over several months throughout the year. The prespawning stage fluctuated during the period of study with notable peaks in April, August and November 1991 and February 1992. The spawning stage in females peaked in July and December 1991, but males released gametes from May 1991 to January 1992.

The continuous spawning from June 1991 to January 1992 was related to fluctuations of phytoplankton levels (microflagellates and diatoms) in Yaldad Bay, suggesting a clear effect of food availability on gametic production. Proliferation of interfollicular material was also related to high levels of food.

Although temperature has been reported as the main factor driving reproduction in marine bivalves, in *A. ater* this factor may be less important for successful gametogenesis and spawning than food availability.

**KEY WORDS:** Mytilidae, reproduction, *Aulacomya*, histology, food supply

### INTRODUCTION

Although the role of endogenous and exogenous factors and their interactions in the synchronization of gamete development and release within a population are still not fully understood (Sasstry 1979, Pearse et al. 1991), the gametogenic phase may be influenced by several environmental factors. The most commonly cited factors are water temperature, food availability and tidal influence (Sasstry 1966, Machell and Demartini 1971).

Many authors have attempted to explain reproductive timing in bivalves primarily in terms of water temperature and its variation with latitude (Loosanoff 1937, Ropes and Stickney 1965, Sasstry 1970, Newell et al 1982, Malachowsky 1988). However more recent studies suggest a close relationship between the reproductive cycle and energy available for growth and gametic maturation (Griffiths 1977, Bayne 1985, Macdonald and Thompson 1986).

Normally eggs and sperm in bivalves are composed primarily of protein and lipid (Pieters et al. 1980); thus, the cyclic pattern of lipid and protein is correlated with the accumulation and shedding of gonadal products. Reproductive activity in *Mytilus edulis* (Linnaeus 1758) is minimal throughout the summer and reserves of protein, lipid and carbohydrate are accumulated in both gonadal and non-gonadal tissues. Glycogen is utilized during autumn and winter for gonadal development (Pieters et al. 1980). In the spring, when glycogen levels are low, spawning occurs as a discrete event or as a series of successive spawnings (Pieters et al. 1980, Lowe et al. 1982). Spawning may also be related to food availability; most bivalves tend to spawn when food is available for developing progeny and for replenishing the energy spent in spawning by adults (Bayne 1976). Thus it is possible that temporal, as well as quantitative and qualitative differences in food supply have a greater influence on the reproductive cycle events (gonadal maturity, spawning and larval growth) than water temperature or latitude (Emmett et al. 1987, Brousseau 1987).

For restocking or mariculture purposes, it is relevant to know the reproductive cycle of any species, and the documentation of the gonadal cycle is one logical step in determining when the spawning and recruitment might occur.

Periodic microscopic examination of gonadal tissue has proven to be a reliable method for determining seasonal gonadal changes in different species (Lauren 1982, Brousseau 1987). Frequently this method has been used to divide the entire cycle into arbitrary categories (developing, ripe, spawning, and spent). The criteria used are based solely on morphological observations.

*Aulacomya ater* (Molina 1782) is a gonochoristic species that lives in shallow waters and forms extensive beds on rocky substrata in South Africa (Griffiths 1977) and South America, between Callao (Perú) and the Strait of Magallanes (Chile) in the Pacific and southern Brazil, Argentina and the Falkland Islands in the Atlantic Ocean (Soot-Ryen 1955, Ageitos de Castellanos 1957).

*A. ater* (Cholga) is a potential economic resource for Chilean mariculture. However, at present, information about the reproductive biology of this species in Chile is scarce, only a few previous papers having been published for South African populations (Griffiths 1977, Griffiths and King 1979a, 1979b, Van Erkom Shurink and Griffiths 1990, 1991, 1992). The present paper describes the gonadal cycle of Chilean specimens, using categories utilized previously by Porter (1974), Keck et al. (1975), Brousseau (1978, 1987), Malachowsky (1988) and Jaramillo et al. (1993). This study also reports data on the effect of temperature and food availability on gametic maturation and spawning in *A. ater* under suspended culture at Yaldad Bay, Chiloé Island, Chile.

### MATERIALS AND METHODS

#### *Temperature and Salinity*

Temperature and salinity were measured at 0.5, 4.0 and 8.0 m depth. The results are expressed as means  $\pm$  SE from these 3 depths.

#### *Environmental Parameters*

Water samples were collected in triplicate from April 1991 to March 1992 by pumping unfiltered seawater from 0.5 and 4.0 m depth for lipids, carbohydrates and phytoplankton analysis. The

results are expressed as means  $\pm$  SE from these depths. The water was screened through a 333  $\mu$ m nitex mesh to eliminate large zooplankton and debris. For particulate lipids and carbohydrates, known volumes of water (1–21) were immediately filtered under gentle vacuum through washed, precombusted, preweighed Whatman GF/C filters (4.7 cm). Filters were stored at  $-20^{\circ}\text{C}$  until the analyses were done. Blank filters were prepared at each collection date and treated in the same manner as the samples.

#### Particulate Carbohydrate

Suspended particulate matter was concentrated by filtering 1–2 L of sea water through a pre-combusted GF/C filter (4.7 cm diameter) for the determination of carbohydrate by the phenol-sulphuric acid method of Dubois et al. (1956), after extraction in hot 5% trichloroacetic acid (TCA) containing 0.1% silver sulphate (Barnes and Heath 1966). Samples and filter blanks were cut into small pieces and homogenized for 1 min in 4 mL 5% TCA with an ultrasonic homogenizer.

#### Particulate Lipid

Particulate lipids were determined by filtering a known volume of water (1–2 L) through a pre-combusted GF/C filter (4.7 cm diameter). The samples and filter blanks were cut into small pieces and homogenized for 1 min in 2 mL chloroform:methanol (2:1) with an ultrasonic homogenizer. After centrifugation at RCF = 1000, the chloroform:methanol extract was dried at  $50^{\circ}\text{C}$  for 5 hrs and the lipid residue charred at  $200^{\circ}\text{C}$  after addition of 0.5 mL concentrated sulphuric acid. Lipids were then estimated spectrophotometrically by the method of Marsh and Weinstein (1966) using tripalmitin as a standard.

#### Phytoplankton

Screened water samples (180 mL) were taken at 4.0 m depth, fixed in Lugol's iodine and the contents were allowed to settle onto 5 mL chambers for examination with an Olympus CK2 inverted microscope.

#### Histological Procedures

Samples of gonads of *A. ater* "Cholga" of both sexes were removed at monthly intervals. A total of 400 specimens were collected from April 1991 to March 1992. The shell length of individuals ranged from 6.0 cm to 9.0 cm. The experimental individuals of *A. ater* were maintained under suspended culture at 2–5 m depth at Yaldad Bay ( $43^{\circ}08'S, 73^{\circ}44'W$ ) on Chiloé Island, Chile.

Gonadal tissue was fixed using Bouin-Hollande (Ganter and Jolles 1970) (picric-formol-acetic + cupric II acetate mixture) for 24 hrs. The samples were then dehydrated, embedded, sectioned at 5 to 7  $\mu$ m and placed on slides using standard techniques (Luna 1968). Tissue was rehydrated using serial increasing ethanol solutions and sections were stained with Hematoxylin-Eosin (Humason 1962).

The histological sections were subsequently examined microscopically, each specimen being assigned to one of the gonadal stages described below.

#### Gonadal Stages

The description of the gonadal cycle represents an attempt to divide the gametogenesis of *A. ater* into different stages. The criteria used for separating these arbitrary stages in what is a

continuous process, are based solely on morphological observations and are rather subjective. All these categories are similar to those used for other bivalves by Ropes and Stickney (1965), Lozada (1968), Griffiths (1977), Brousseau (1978, 1987), Emmett et al. (1987), Malachowsky (1988) and Jaramillo et al. (1993).

#### Gonad Index

Monthly samples of 15 gonads (male and female) were used for Gonad Index (GI). Only mantle and mesosome were considered for this index. At this sample size the standard error of the mean of GI remained below 9% which was regarded as highly precise. The following relationship was used to determine the gonad index:

$$GI = \frac{(\text{fresh wet weight of gonad})}{(\text{fresh wet weight of soft parts})} \times 100$$

This wet weight technique is regarded as a rapid and quantitative estimate of gonadal development in field studies. The dissection of the gonadal tissue in bivalves is complicated by the fact that this tissue cannot be completely separated from the rest of the body and must be weighed together with the interfollicular tissue. We use easily recognized gonadal tissue like mesosome and mantle. This technique however should be supported by histological analysis to detect changes in gonad structure which may affect weight;

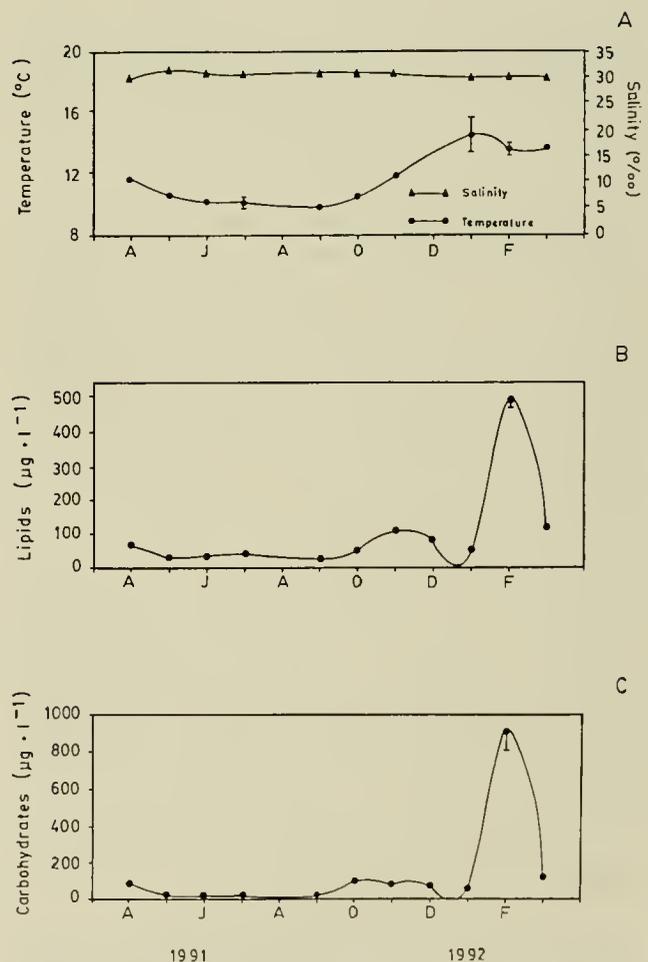


Figure 1. (A) Mean seasonal fluctuation of mean temperature and mean salinity, (B) particulate lipids, (C) carbohydrates, in Yaldad Bay. (Monthly means  $\pm$  SE).

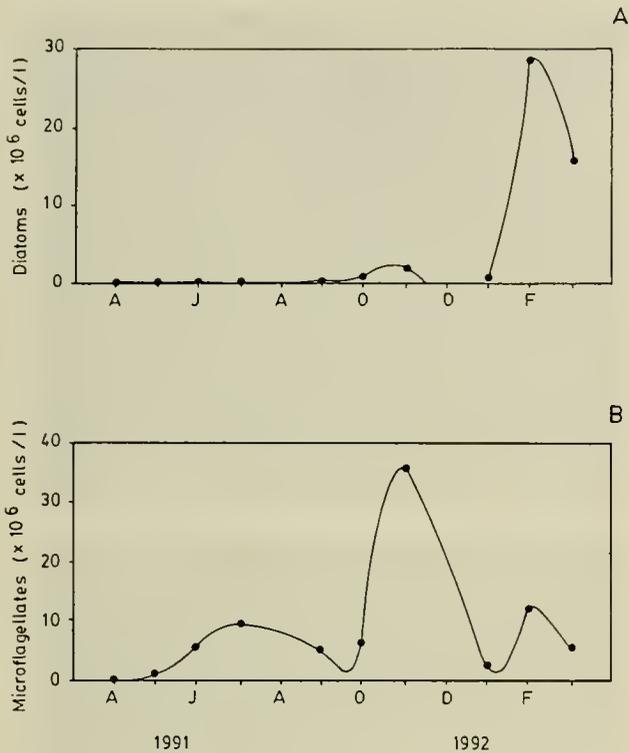


Figure 2. (A) Seasonal cycle of the diatoms and (B) microflagellates in Yaldad Bay.

i.e., accumulation of reserve material (Griffiths 1977). The high values of GI are considered to be coincident with gonadal maturity. Minimal values following high values are regarded as an indicator of spawning (Akaboshi and Illanes 1983).

Gonad index is affected by the accumulation and release of gonadal material as well as the utilization of stored energy products during winter months. It is therefore necessary to realize that minimal seasonal variation in wet weight of mesosome or mantle may not only represent increasing gonadal development, but also can be due to a proliferation of interfollicular material.

RESULTS

Environmental Parameters

Mean temperature increased from a minimum value of 10°C during winter months (July–August) to a maximum of 15°C in the summer (Fig. 1A).

Mean salinity was high throughout the year, with values around 30‰ (Fig. 1A).

Particulate lipids showed a small peak during November 1991 which reached values of 100 µg/L. The main peak was observed in summer of 1992 (February), with a value of 520 µg/L. Lower concentrations of lipids were observed during the winter, with values reading 16 µg/L (Fig. 1B).

Particulate carbohydrates showed a very similar seasonal cycle to that shown by the lipids, with a small peak in October 1991 which was maintained until December. The highest value was observed during February 1992, with a concentration of 900 µg/L (Fig. 1C).

In order to estimate the composition of the living fraction of the food supply available to *A. ater*, a quantification of the main

components of the phytoplankton (diatoms and microflagellates) was carried out. Values peaked in February 1992 with values of 30 × 10<sup>6</sup> cells/L (Fig. 2A). This peak was mainly composed of the diatom *Schroderella delicatula* (59%). The microflagellates (Fig. 2B) showed a maximum in November 1991, when the diatom concentration was at its minimum. Smaller peaks in abundance were observed during winter and late summer.

Reproductive System

Females may be distinguished easily from males by their dark-brown gonad containing purple patches. The testis of the male is yellow to white, depending on its state of development. The gonads are formed by numerous tubes ramified through the mesosome and visceral mass and extending into the mantle.

Gonad Index

Gonad index (GI) was high in April and May (Fig. 3A), lower in June and high again in July. The highest GI was registered in August and the lowest value during September, suggesting a spawning period in August–September 1991 (Fig. 3A). During

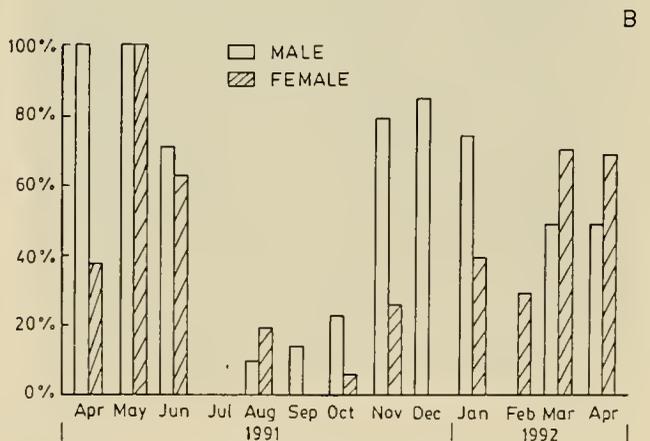
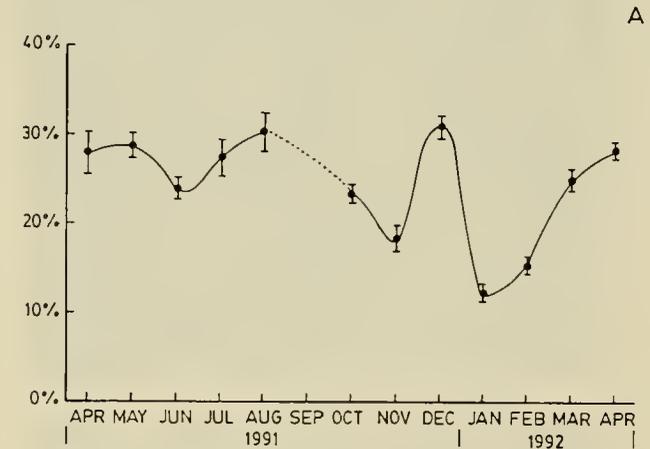


Figure 3. (A) Seasonal fluctuations of Gonad Index.

$$GI = \frac{\text{fresh wet weight of gonad}}{\text{fresh wet weight of soft parts}} \times 100$$

(B) Percentage frequency of gametogenic phase developing of *Aulacomya ater*.

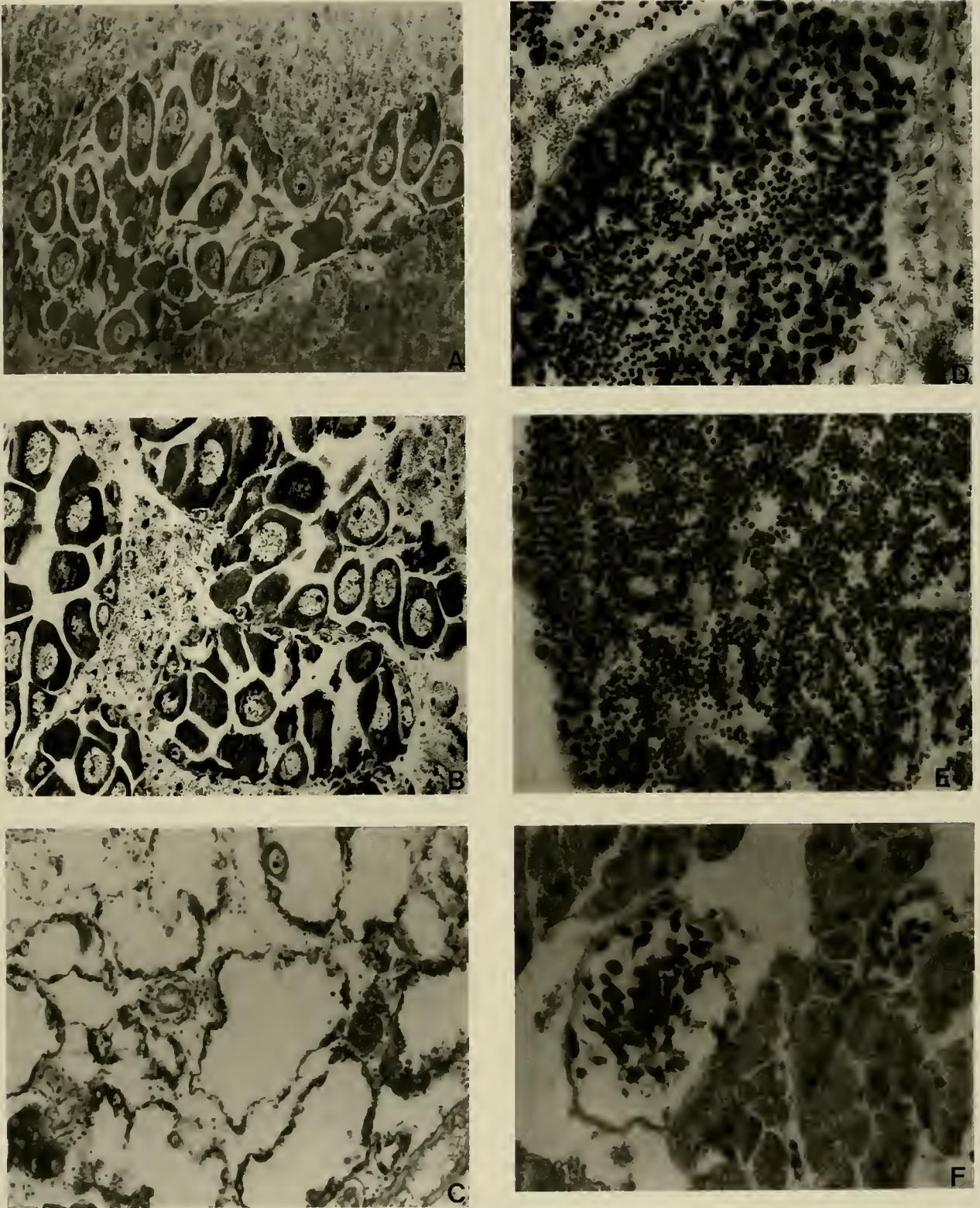


Figure 4. Photomicrographs of gonadal sections of *Aulacomya ater* (A) developing female (500 $\times$ ), (B) spawning female (500 $\times$ ), (C) postspawning female (256 $\times$ ), (D) developing male (250 $\times$ ), (E) spawning male (250 $\times$ ), and (F) postspawning male (250 $\times$ ).

August 1991 the values for spawning in male and female were decreasing. In addition, settlement of spat was observed during September, confirming the August–September spawning.

From September to November 1991 the GI was fluctuating,

which can represent a partial spawning. Another peak of GI was observed in December. The low values of GI of January and February probably indicate a new spawning time, but spat were not registered during this period.

### Gonadal Cycle

The gonadal histology reveals the variation in the amount of interfollicular material during both breeding and the non-breeding period.

Photomicrographs of the different gonadal stages observed in *A. ater* are presented in Figure 4.

In the female the *developing stage* was characterized by follicles showing oocytes in different stages of development on the follicle wall, the size of these oocytes fluctuated between 67–70  $\mu\text{m}$  (Fig. 4A).

The *spawning* ovary exhibited distended follicles with many mature oocytes (75  $\mu\text{m}$ ), that appeared to be free within the follicular lumen, some of these oocytes were gradually discharged except for redeveloping oogonia or peduncled oocytes lining the wall (Fig. 4B).

In the *postspawning stage* the follicles were empty except for some oogonia lining the walls (Fig. 4C).

In the male the *developing stage* was characterized by some isolated follicles and a few stem cells. Spermatogonia, spermatocytes and early spermatids were present in the center of the lumen. The differentiation into spermatozoa follows the proliferation of spermatocytes and spermatids. (Fig. 4D).

In the *spawning* testis the follicles were filled with dense packs of spermatozoa. The mature spermatozoa were gradually discharged and new spermatozoa replaced those previously spawned (Fig. 4E).

The *postspawning stage* was characterized by follicles containing a few sex cells (Fig. 4F).

Histological observations showed a high percentage of males and females spawning in July 1991 (Fig. 5A). The developing stage of the females fluctuated greatly during the entire study period (Fig. 3B). Proliferation of interfollicular material occurred during the peak values, where the gonad was thin and transparent so that the developing follicles could be easily distinguished because gametogenesis in both sexes usually occurs soon after gametic release. In males, the developing stage was observed from April to June of 1991, and from October 1991 to February 1992.

In females, the spawning stage was observed during the entire study exhibiting peak values during June, September and December of 1991. There was a rapid regeneration of the gonad and the interfollicular material was utilized during the rapid and continual gametogenesis which took place. However a sudden total release of all gametes was not observed so that the gonad usually appeared partially spawned. In males, the spawning stage was observed from May 1991 to February 1992 with peak values in July 1991 and January 1992, with continual gametogenesis throughout the breeding season. A high proportion of the sampled population (70% male and 100% female) was observed to be in a spent or resting phase during February and March 1992 (Fig. 5B).

### DISCUSSION

In Yaldad Bay, *A. ater* "Cholga" shows a continuous gamete release taking place over several months with a peak period in spring. A similar pattern was described by Griffiths (1977) for this species in South Africa.

The abundance of food has been generally associated with the breeding period of marine bivalves, probably because mussels do not store large reserves in the mantle and have an immediate gonadic regeneration with high food availability (Griffiths 1977). The effect of food on gonadic production is clearly observed in *A.*

*ater* of Yaldad Bay. The developing is observed in 2 peak periods (April and December 1991). The April peak occurs 1 month after the period of enhanced food availability (microflagellates), but the December peak is coincident with high food availability (lipids, carbohydrates, diatoms and microflagellates).

Thus probably during the autumn months (April to June) when the animals remain in a reduced gonadic condition, the available protein, lipids and carbohydrates may be accumulated in both gonadal and non-gonadal tissue. The fraction accumulated in the gonadal tissue (interfollicular material) is immediately utilized, producing an increase of spawning individuals. In July, October and December, when the spawning stage is beginning, reserves could be utilized for the synthesis of gonadal tissue (prime lipid and protein) supplemented by high levels of microflagellates.

In October, when high levels of food were present at Yaldad Bay, the gametogenesis was extremely rapid and mature ova were always present. Proliferation of interfollicular material also takes place at this time, but in small quantity, forming only thin layers between the follicles, insufficient to thicken the mantle which remained thin and gelatinous in appearance, as cited for *M. edulis* (Gabbott 1976) and *Choromytilus meridionalis* (Griffiths 1977).

From June to August, when high levels of microflagellates

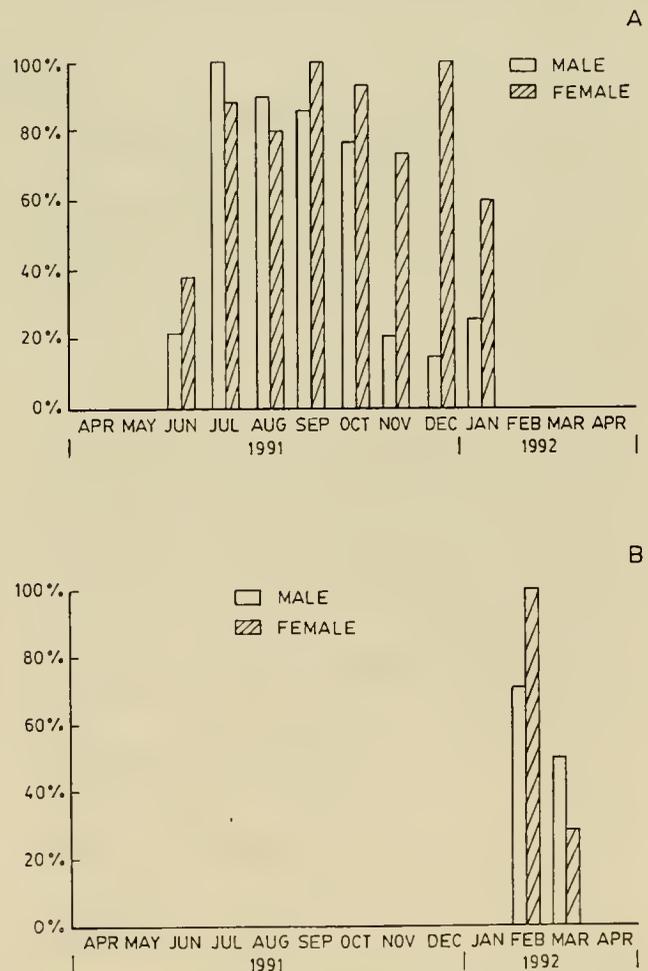


Figure 5. Gametogenic phases of *Aulacomya ater*, the values represent the percentage frequency in each stage. (A) spawning, (B) postspawning.

were available, an increasing number of individuals showed spawning stage. Thus it is possible that a continuous spawning, in a small percentage of *A. ater*, may occur during winter. The highest percentage of individuals releasing gametes was observed during September and October. Late October was characterized by a high increase in food availability, which produced a continuous regeneration of the gonad which was associated with both a frequent absence of a resting period and absence of sudden total release of all gametes. Thus the ovary usually contained high percentage of mature ova, facilitating continuous spawning from June 1991 to January 1992 in a variable number of individuals of *A. ater*. Carreño and Avilez (1977) described continuous spawning with peak periods in autumn and spring for *A. ater* of Isletilla (Chiloé), a location slightly to the north of Yaldad Bay. A similar phenomenon was reported by Henriquez and Olivares (1980) for a northern population of *A. ater* (Mejillones Bay). However, a spent stage (resting phase) is observed in Yaldad during February and March 1992, which is in agreement with results of Henriquez and Olivares (1980) for Mejillones. This gonad depletion and reabsorption of gametes may be considered as the end of the breeding season.

Bivalves tend to spawn during periods when food is available to ensure adequate nutrition for the planktotrophic larvae and for replenishing the energy of adults spent in spawning (Bayne 1976).

Continuous spawning in *A. ater* from June 1991 to January

1992 is related to fluctuations of phytoplankton abundance in Yaldad. There were not always high concentrations of microflagellates throughout the year, rather there was a variable quantity from May to September and a marked peak from October to January.

Nevertheless, temperature has been reported as the main factor driving reproduction in marine bivalves. Lozada (1968) reports gametic release during autumn months for *A. ater* and suggests that this may be caused by stimuli other than temperature, because average temperature was relatively low during these months. Wolff (1988) suggests that although high temperature may stimulate gonad maturation and spawning in the scallop *Argopecten purpuratus* it might be less decisive than food availability.

The extended spawning observed in *A. ater* of Yaldad Bay can be correlated with the maturation condition of the gonads and a favorable external stimulus. Thus we can accept that spawning is induced by a combination of internal and environmental factors and that their interaction may vary seasonally, producing annual variations in onset and intensity of spawning.

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## EFFECTS OF HANDLING ON FEEDING, ACTIVITY AND SURVIVAL OF RED KING CRABS, *PARALITHODES CAMTSCHATICUS* (Tilesius, 1815)

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**ABSTRACT** Crab pots are used to commercially fish for king crab in Alaska. A large number of female and sublegal-sized male red king crabs are caught in this male-only fishery. Before being returned to the sea, these crabs suffer aerial exposure, crushing, and deck and water impacts. This study examined the potentially deleterious handling effects of simulated commercial fishing procedures on female and sublegal male red king crabs. A total of 135 crabs were distributed equally into 5 treatments: handled once (receiving deck impact and a 3 m fall to sea water), handled twice, handled three times, modified handling (no deck impact and return to the sea via a ramp), and controls (unhandled). Crabs were categorized in 3 groups: ovigerous females, juvenile females, and sublegal males. After receiving handling treatments, the crabs were maintained for 4 months while mortality, feeding rates, righting response, and bacterial infections were monitored. Body damage increased significantly with increased handling. One crab died within 24 hours of the first handling treatment. However, there were no significant differences in long-term mortality, feeding rates, righting responses or bacterial infections among the 5 treatments of 3 sex groups. Handling of red king crabs during commercial crabbing activities may have fewer detrimental effects than has been reported for other crustaceans.

**KEY WORDS:** King crab, handling effects, mortality, feeding, activity

### INTRODUCTION

Both male and female red king crabs, *Paralithodes camtschaticus* (Tilesius 1815), are caught in pots in the commercial fishery in Alaska, but only legal-sized males may be retained. During the harvest and sorting operations, a large number of female and sublegal-sized males are exposed to air (and the resulting desiccation and large temperature gradients), are crushed by the catch or gear, damage each other by pinching, and receive impacts when dropped on deck and when returned to the sea.

Handling of king crabs during sorting and deck operations often results in body damages which include limb injuries. Severe damage of a leg may cause subsequent autotomy, which results in limb loss of the leg at a site near its base. Juveniles are particularly vulnerable to limb loss (Edwards 1972, Kurata 1963, Niwa and Kurata 1964). Little quantification exists for limb loss and mortality of red king crabs resulting from pot capture and commercial crabbing activities. However, the immediate mortality (47%) of red king crabs captured by bottom trawls was high (Stevens 1990). Similarly, trawl-caught Norway lobster (*Nephrops norvegicus*) had high (40%) short-term mortality (Chapman 1981).

A field survey using king crab pots in Kodiak, AK reported that 75% of king crabs caught were female, and, of the males captured, 26% were sublegal size (Blau 1988). This means that 81.5% of the king crabs captured had to be returned to the sea. The sex ratio in the catch in Bristol Bay varied from year to year. The male:female ratio in a 1991 survey was 47:53. In the 1992 survey, although the male:female ratio was high (70:30), and 62% of males caught were legal-sized (>165 mm CL) (Byersdorfer and Watson 1992), as high as 57% of the red king crabs caught had to be returned to the sea.

Before they are released, these discarded female and sublegal-sized male red king crabs are exposed to desiccation in air and often to large sea water and air temperature differences. They may also crush each other, and sustain damage when dropped on the deck and into the sea during sorting and release operations. These

procedures have been suspected of increasing mortality and perhaps playing a role in depleting red king crab populations.

Other potentially serious consequences of sorting and handling are impact wounds, contusions, autotomy, and secondary infections of these wounds. The amount of physical trauma received from impact with the vessel deck, and that crabs later endure when dropped from commercial vessels at 2-3 meters above the water surface, is unknown. Likewise, the number of times that immature and female king crabs are recaptured by the commercial fishery is poorly known.

Ship design, pot structure and handling protocol vary greatly among commercial crabbers in Alaska. Crabs captured in cone-shaped pots may receive a greater impact if crabs are released onto the deck from a drawstring on the underside of the pot. Crabs caught in standard rectangular pots are usually dumped out of a side door onto the deck or into sorting bins, with the pot being tipped over by a hydraulic arm (pot launcher). Variation in handling stress also occurs on the same vessel. Full pots routinely have their catch dropped onto the deck or into sorting containers. A pot with only a few crabs may instead have its crabs individually retrieved from the pot and returned to the sea. Crabs caught in pots containing many crabs may be exposed to more crushing weight and longer sorting times. The height that sublegal and female crabs are dropped from the ship's deck when returned to the sea varies with ship design, how laden the ship is, and sea conditions. If crab are thrown back to the sea over the rail, most commercial vessels have a drop distance of at least 2 meters.

Prior investigations of handling effects on Dungeness crabs in southeastern Alaska demonstrated that increased handling resulted in higher mortality, culminating in 100% mortality after crabs were handled 4 times with techniques simulating those used in the commercial fishery (T. Shirley, unpublished data). The mortalities were not due to acute injuries, but rather occurred over a 3 month period following the handling. The average number of missing limbs and percentage of the population with missing limbs increased as the Dungeness crab season progressed (Shirley and

Shirley 1987). Because red king crabs are larger and heavier than Dungeness crabs, and would be expected to have fewer adaptations to aerial exposure and impacts because of their subtidal life style, the effects of handling may be more deleterious. Recapture rates of sublegal and female king crabs are unknown, but should vary with the density of pots, the length of the season, and the number of times that pots are redeployed.

This study examined the effects of sorting and handling by the commercial fishery on sublethal stress and survival of sublegal and female red king crabs. We examined: 1) the effects of handling on survival; 2) the effects of handling on body damage which includes limb damage and autotomy; 3) the effects of handling on feeding rate; 4) the effects of handling on activity; 5) the effects of repeated handling on these indexes; 6) the causative mechanisms or agents which result in mortality (specifically, whether bacterial infections can be implicated); and, 7) whether handling impacts can be ameliorated by alteration of handling techniques.

## METHODS

Sublegal male and female red king crabs were collected near the laboratory in Auke Bay and Barlow Cove, Alaska, with commercial and sport pots, handled gently, and maintained in sea water during transport to the laboratory. Within the laboratory, crabs were kept in tanks with flowing seawater from a -30 m intake and fed a mixed diet of fish, squid and mussels ad libitum. All crabs were acclimated to laboratory conditions for at least 2 weeks prior to experimentation.

Each crab was individually numbered with a numbered cinch tag attached to the basis of the right third walking leg. Sex, wet weight, and carapace length were recorded for each crab. No new autotomy resulted from initial capture and during maintenance in the laboratory. Crabs with missing leg(s) were not used for the experiment. The experiment consisted of 5 treatments, and 27 crabs were used in each treatment: 9 ovigerous females, 9 non-ovigerous females, and 9 sublegal males. Crab sizes were selected so as to have similar-sized crabs within each treatment, and the placement of crabs into each treatment was determined by randomized block design. A total of 135 individual crabs of the appropriate sex and size were used for the experiment.

Deck impacts and water impacts were studied by treating crabs in a manner similar to that experienced onboard commercial vessels. The handling procedures for the 5 treatments were as follow.

*Treatment 1.* Handled once. Twenty-seven crabs were placed in a simulated commercial pot (approximately one-half size, 92 \* 92 \* 45 cm, but of similar box shape); the pot stood on a height of 60 cm and subsequently tilted at a 45° angle; the door of the pot was opened and crabs fell (dumped) into an empty tank; the crab tangled on the pot-mesh were shaken to cause them to fall and no crab was pulled out by hand; crabs were then dropped from 3 meters height into seawater on to their dorsal surface.

*Treatment 2.* Handled twice. All crabs in this treatment received the same handling as Treatment 1. Three days after the first handling, crabs were repeatedly handled in the same way as the first handling, except that the crabs were dropped into water from 3 meters height onto their ventral surface. This permitted crabs in this group to experience one dorsal water impact and one ventral water impact.

*Treatment 3.* Handled three times. All crabs in this treatment received the same handling as Treatment 2. Three days after the second handling, crabs were repeatedly handled in the same way

as during the first handling, i.e., crabs were placed in a pot, dumped into a empty tank from 60 cm height, and dropped from 3 meters height into sea water onto their dorsal surface. For water impact, crabs in this treatment experienced two dorsal impacts and one ventral impact.

*Treatment 4.* Modified handling. Twenty-seven crabs were placed in a pot; the pot stood at 60 cm height from the deck; when the door opened, crabs fell into a tank filled with sea water of 40 cm depth (the distance between the pot and water surface was 20 cm); then crabs slid on their ventral surface into sea water from a 45° tilted entry of 3 meters height rather than being dropped.

During the handling of these 4 treatments, water temperature varied between 7.8 and 8.6°C and air temperatures varied between 7.6 and 15.3°C. Aerial exposure time for the last crab returned to the water varied from 10–14 min. in Treatment 1 and Treatment 3.

*Treatment 5.* Control. This group received no handling or aerial exposure after the initiation of experiments, other than that used for determining weights and measurements.

Crabs from all treatments were returned to laboratory holding tanks for examination. Crabs used for feeding measurements and crabs undergoing molting were kept in individual compartments.

Body injury and limb autotomy, if any, were recorded for each crab immediately after each experimental treatment. Mortality was recorded daily.

One day after treatment, the righting time which a crab required to turn over when placed on its back under water on the bottom of the tank was recorded for each crab. Righting time has been found to be a sensitive indicator of general well-being of many marine invertebrates, as an integrated coordination of muscles and sensory perception is required for rapid righting (Shirley and Stickle 1982). Righting time was measured weekly until the twelfth week.

Feeding rates were measured by placing known weights of food into each crab container and weighing all food remaining in the chamber 24 hours later. Feeding rates were measured twice a week for a subset of 9 crabs in each treatment (3 of each of the subgroups: sublegal males, non-ovigerous females and ovigerous females) until the thirteenth week.

At the termination of the experiments (4 months after experimental treatments), wet weights and carapace lengths were recorded for all crabs.

Blood samples were collected from crabs used in feeding determinations (9 crabs in each treatment) at the end of the experiment to examine the incidence and intensity of bacterial infection. Hemolymph samples were obtained by puncture of the periarthrodial membrane at the base of the third walking leg with a sterile 1 mL syringe fitted with a 20G1½ needle. A protocol developed by the Alaska Department of Fish and Game was used (Dr. T. Meyers, pers. comm.). Blood smears were made by expressing a drop or two of hemolymph onto a glass slide and pushing a second slide to drag the blood by capillary action and produce a uniform thickness smear. Slides were air-dried, stained with Diff-Quik, and examined with phase contrast optics. Bacterial rods were counted in 50 randomly selected fields on each slide.

A  $\chi^2$  test for differences in probabilities was used to test for differences in mortality and injury among treatments (Conover 1980). Also the  $\chi^2$  analysis was used to test for differences in incidence and intensity of bacterial infection among treatments. Two-way ANOVA with equal cell numbers was used to test for differences in feeding rates (Kleinbaum et al. 1988). Before testing, the data were standardized to grams of food consumed per 100

grams of crab (wet weight) for 24 hours. Similarly, two-way ANOVA was used to test crab righting time among treatments and groups.

## RESULTS

Mortality was uniformly low in all experimental groups. A total of 18 crabs of the 135 crabs used in the experiment died over the 4 month study; 6 of the mortalities were due to experimental error (Table 1). There were 2 mortalities of unknown causes in each of the handling treatments and the control group, except for the treatment group which was handled twice, which had 4 mortalities. In the treatment handled once, one crab died within 24 hours of the handling treatment and was considered to be immediate mortality. All other unknown mortalities were considered delayed mortalities. There were no significant differences in mortality among the 5 treatments (Table 1).

Damage increased with an increase in handling. A significant difference in damage among the treatments occurred for all damage types combined, and for the rostrum and spine damage (Table 2). Spines were damaged more commonly than the legs, rostrum or carapace; 89% of the crabs handled 3 times had damaged body parts, either legs, rostrum, carapace or spines; while only 26% of the crabs handled once had damage to body parts. Crabs in the modified handling treatment (without deck impact and returned to

TABLE 1.

Mortality of red king crabs in handling experiment of four months duration (September 15 to January 15).

Date	Treatment				
	1	2	3	4	5
9-15	1				
9-28				1 <sup>a</sup>	
10-17				1 <sup>b</sup>	1 <sup>b</sup>
10-17					1
10-23	1 <sup>c</sup>				
10-27			1	1	
10-30					1
12-02	1				
12-15				1	
12-25		1			
12-28		1			
12-31			1		
1-04	1 <sup>d</sup>				
1-05		1			
1-07		1			
1-10		1 <sup>e</sup>			
Total	2 (+2)	4 (+1)	2	2 (+2)	2 (+1)

<sup>a</sup> Crab fell out of tank.

<sup>b</sup> Water in the tank dried out.

<sup>c</sup> Crab injured by falling tank divider.

<sup>d</sup> Two legs of the crab severed, perhaps due to cannibalism.

<sup>e</sup> Water flow accidentally stopped.

The coding of the treatment is: 1 = handled once, 2 = handled twice, 3 = handled three times, 4 = modified handling, 5 = control. There were only two mortalities of unknown cause in each of the treatments 1, 3, 4, and 5, and four unknown mortalities in treatment 2. The numbers in the brackets indicate the mortalities due to experimental error. Chi-square test:  $df = 4$ ,  $\alpha = 0.05$ , statistics  $T = 1.46 < X_{0.95} = 9.488$ . There is no significant mortality difference among the five treatments.

TABLE 2.

Number of crabs with damage induced by handling.

Treatment	Any part	Leg	Rostrum	Carapace	Spines
1	7	1	2	1	3
2	12	0	3	0	11
3	24	3	5	0	19
4	1				1
5	0				
Significance	**		*		**
Total	44	4	10	1	34

The coding of the treatments is the same as in Table 1. The damage (broken parts) was examined immediately after each handling. Chi-square test of the effect of treatments excluding the control ( $df = 3$ ,  $X_{0.95} = 7.815$ ,  $X_{0.99} = 11.345$ ): any part damage,  $T = 43.88$ , significant difference; leg damage,  $T = 6.23$ , no significant difference; rostrum damage,  $T = 9.53$ , significant difference; carapace damage,  $T = 3.03$ , no significant difference; spine damage,  $T = 34.85$ , significant difference.

water by means of a slide) had the least damage, approximately 4%.

Feeding rates did not differ significantly among the treatments or by sex group, after feeding rates were standardized to grams of food consumed per 100 grams of crab wet weight per 24 hours (Fig. 1). Average feeding rates of the different treatments varied between 4.80 and 5.25 g/100 g crab · day<sup>-1</sup> and overall averaged 5.04 ± 1.57 (mean ± standard deviation) g/100 g crab · d<sup>-1</sup>. No significant differences in average feeding rates occurred among the treatments over time (Fig. 2).

Righting time did not differ significantly among the treatments or by sex groups (Fig. 3). However, the righting time increased over time for all treatments. A linear regression, Righting time = 1.6 + 0.01 \* Days after handling, was fitted to the data. This regression had a correlation coefficient  $r = 0.93$ , and a significant association between righting time and the number of days after handling ( $n = 9$ ,  $p = 0.0003$ ). The slope of 0.01 was significantly larger than zero ( $t = 6.71$ ,  $p = 0.0002$ ). According to the regression equation, during the 3 months after handling, the av-

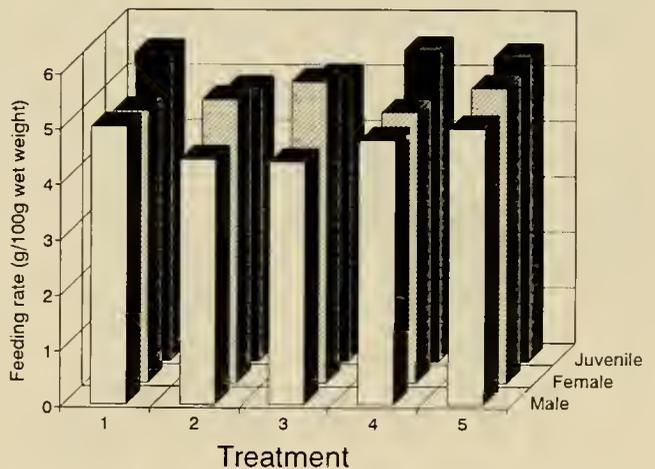


Figure 1. Average feeding rate of red king crabs in handling experiments. Codes for treatment are: 1 = handled once, 2 = handled twice, 3 = handled three times, 4 = modified handling, and 5 = control. There was no significant difference of feeding rate among treatments and sex groups (ANOVA,  $p > 0.05$ ).

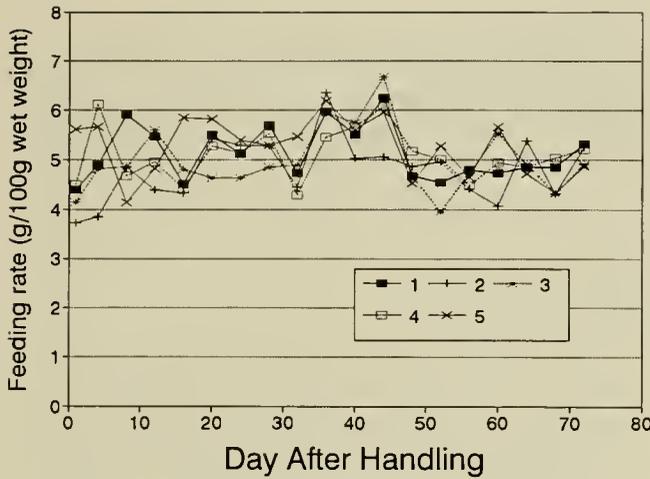


Figure 2. Feeding rate of red king crabs over time. The coding of the treatments is the same as in Figure 1. The data were the average of 9 crabs in each treatment, including sublegal males, ovigerous females, and juvenile females. No significant change of feeding rate over time was detected (ANOVA,  $p > 0.05$ ).

crab righting time increased from 1.6 seconds to 4.5 seconds, i.e., 56%.

Bacterial counts were low and not related to treatment (Table 3). Only 1 out of 9 crabs sampled in each of the experimental treatments contained bacteria in their hemolymph.

DISCUSSION

In Alaska, crab pots are the only legal commercial fishing gear for red king crab. In comparison to other fishing gear (e.g., trawls) pots have many advantages: they are inexpensive, fish unattended, have minimal bycatch, and are suitable for most bottom types and depth ranges. In addition, their requirements for engine power and deck equipment are modest. However, a large number of female and sublegal-sized male (<178 mm or 7 inches in carapace width, or <165 mm or 6.5 in CL, depending on the management area) are incidentally caught in this male-only fishery (Otto 1985, Blau 1988, Byersdorfer and Watson 1992).

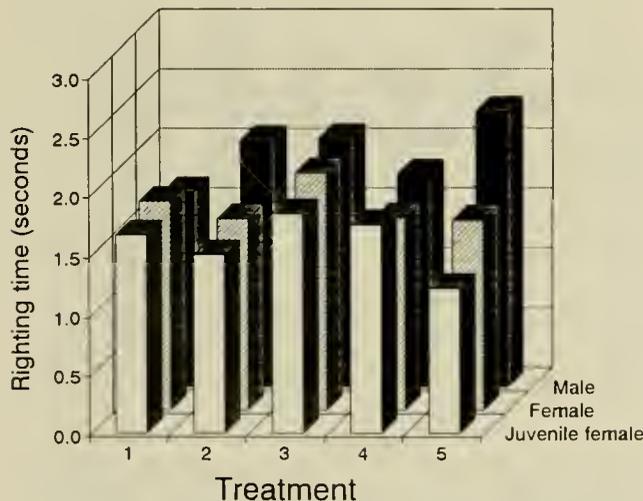


Figure 3. Average righting time of red king crabs. The coding of the treatments is the same as in Figure 1. There was no significant difference among treatments and sex groups (ANOVA,  $p > 0.05$ ).

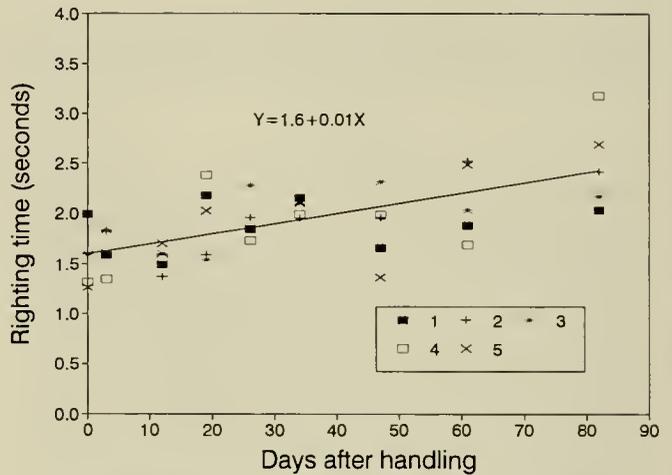


Figure 4. Righting time of red king crabs over time. The coding of the treatments is the same as in Figure 1. The data were the average of 12 crabs for each treatment, including sublegal males, ovigerous females, and juvenile females. There was no significant difference among treatments and sex groups. However, the righting time increased over time for all treatments. A linear regression was fitted to the data.

Red king crabs, especially juveniles, are vulnerable to autotomy (Edwards 1972, Kurata 1963, Niwa and Kurata 1964). The Alaska Department of Fish and Game conducted experimental fishing with pots in 1991 and reported that 2% of crabs were injured and 0.1% died immediately after handling (Byersdorfer and Watson 1992). There was no report on the incidence of handling-induced injury or mortality during commercial fishing with pots. The immediate mortality (47.3%) of king crabs captured by commercial sole trawls was high (Stevens 1990). Exposure to cold air reduced vigor, feeding rates and growth, and also caused limb autotomy and mortality in ovigerous red king crabs and Tanner crabs (Carls and O'Clair 1990). Severely exposed king crabs often died during ecdysis.

Crabs and lobsters returned to the sea by fishermen had increased injury, reduced growth, and increased mortality (Brown and Caputi 1985, Simonson and Hochberg 1986). When the time that rock lobster were exposed to sunlight and air increased, the percentage of inactive animals and loss to predators increased, and the descent rate through the water column and the recapture rate decreased (Brown and Caputi 1983). Sixty to seventy percent of discarded spanner crabs (*Ranina ranina*) with one or more dactyls broken off by handling during commercial fishing died within 50 days (Kennelly et al. 1990). Instantaneous crab mortality esti-

TABLE 3.

Bacteria counts at the end of red king crab handling experiment.

Treatment	1	2	3	4	5
# of crab with bacteria	1	1	1	1	1
# of bacteria/crab	1	1	1	5	2

The coding of the treatment is the same as in Table 1. Nine crabs were sampled from each treatment. Bacteria were counted within two random selected transection cross the slide, which is about 160 fields of observation. Only one crab was found with bacteria for each treatment sample; only one bacterium was observed in each of the three crabs, and two and five bacteria in the other two crabs.

mated from measuring declawing wounds of stone crabs harvested by 4 commercial fisherman ranged from 23 to 51% (Davis et al. 1978).

After their first year of life, red king crabs are exclusively subtidal inhabitants; consequently, they would be expected to have few adaptations to the impacts, abrasion and crushing associated with fishing and handling. The greater size and heavier weight of red king crabs in comparison to other species (e.g., Dungeness crabs) should tend to exacerbate the potential effects of handling incurred during sorting activities.

In our study, ovigerous and non-ovigerous females and sublegal-sized male red king crabs displayed few acute or chronic responses to laboratory handling treatments designed to mimic capturing, sorting, and discarding operations in the commercial fishery. There was no significant difference in mortality among the 5 treatments for 4 months after experimental treatments. Similarly, no significant differences were found in feeding rates, activity (righting times) or the incidence and intensity of bacterial infections, among unhandled crabs, crabs with modified handling, crabs handled once, twice and three times. Significantly increased damage to body parts resulted from increased handling, but the damage did not result in increased mortality or physiological correlates of decreased viability.

We hesitate to infer from the results of our experiments that handling during commercial crabbing activities (e.g., crushing, pinching, autotomy, aerial exposure, deck and water impacts) causes little or no mortality to sublegal-sized and female red king crabs. The handling that crabs received in our experiments was probably conservative in comparison to that received during commercial crabbing activities. In our experiments, the crabs were not on the deck of a pitching and rolling crab vessel during late fall and winter when crab fisheries occur. Our experimental crabs received minimal aerial exposure, with the average time of exposure being only a few minutes, and a minimal temperature gradient. Little

crushing was involved in our study, as only 27 sublegal-sized crabs were contained in each crab pot during dumping and sorting. No larger and heavier, legal-sized males were included in our treatments, which might have contributed to crushing effects; commercial crab pots often contain large numbers of legal crabs.

On the other hand, our results indicated that if crabs were normally handled as in our experiments, the handling would not induce high mortality and serious physiological impacts on crabs. In other words, the results did not support the assumption that handling was a critical factor contributing to the decline of red king crab abundance.

The effects of commercial crabbing operations on mortality of non-targeted portions of crab populations have been examined for only a few crab species. Those species that have been examined generally had alarmingly high levels of mortality of non-target sex and size groups associated with capture. Additional studies, particularly field aspects involving the quantification of damage and mortality of red king crabs received during commercial crabbing operations, are warranted.

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## ESTIMATION OF THE NATURAL MORTALITY RATE OF GREEN TIGER PRAWNS *PENAEUS SEMISULCATUS* (DE HANN, 1844) IN KUWAIT WATERS USING RELATIVE ABUNDANCE DATA

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**ABSTRACT** A method utilizing relative abundance data from research surveys during the closed fishing season was proposed to estimate the natural mortality rate of a population. The natural mortality rates for female, male and sexes combined of the green tiger prawn, *Penaeus semisulcatus*, in Kuwait's waters were estimated to be 0.23, 0.44 and 0.33 month<sup>-1</sup>, respectively, by the proposed method with corresponding confidence intervals of 0.11-0.36, 0.35-0.53, and 0.23-0.43 month<sup>-1</sup> constructed by the bootstrap and percentile methods. The bootstrap simulation indicated that the estimation performance of the proposed method as an estimator for natural mortality rate was satisfactory. The natural mortality accounted for 46, 77 and 61% of the total mortality estimated by the method of decline in CPUE over time for female, male and sexes combined green tiger prawn, respectively. Estimates of natural mortality rates for green tiger prawns using different methods showed that the natural mortality rate for males is higher than that for females. With the estimated natural mortality by the proposed method and the estimated total mortality by the method of decline in CPUE over time, a cohort of green tiger prawns in Kuwait's waters would not survive through 2 successive fishing seasons.

**KEY WORDS:** Natural mortality, shrimp, closed season, relative abundance

### INTRODUCTION

The natural mortality rate is an important parameter of most population assessment models such as age-based or length-based cohort analyses (Gulland 1965, Pope 1972, Jones 1981), and it greatly affects the output of the population estimates (Sims 1984, Lai and Gallucci 1988). Therefore, a reasonable input of natural mortality for these population assessment models is a key point in obtaining reliable population estimations. Natural mortality is a difficult parameter to estimate, however, especially for a heavily exploited population. Mark-recapture methods (e.g., Chapman 1961, Paulik 1963, Hearn et al. 1987, Farebrother 1988) and regression or empirical models of natural mortality on some life history parameters (e.g., Gunderson 1980, Pauly 1980) are the most frequently used methods for estimating natural mortality of an exploited population. Because of the difficulty in conducting tagging experiments for crustacea and the low reliability for tag recovery, the application of mark-recapture methods has been highly limited in population studies. Thus, regression models such as Pauly's method (Pauly 1980) become more attractive for their simplicity.

Green tiger prawns, *Penaeus semisulcatus* De Haan 1844, rank as Kuwait's most important commercial species of shrimp. Total annual landings from the 1980/1981 through 1989/1990 seasons ranged from 1,515 to 5,125 tons with green tiger prawns accounting for as much as 98% of the catch (Siddeek et al. in press). Although found throughout Kuwait's territorial waters, the primary distribution of green tiger prawns in the western Gulf occurs from Kuwait Bay to Bahrain (Farmer and Ukawa 1986). Tagging experiments in Kuwait found no evidence of international movements by local green tiger prawn stocks (Shoushani et al. 1984). Spawning occurs throughout the year with a maximum activity from January to April (Mohamed et al. 1981), and with a second peak in late autumn (van Zalinge 1984). The offspring of the spring and autumn spawning stocks were usually denoted as spring

cohort and autumn cohort, respectively (van Zalinge 1984). Post-larvae of the spring cohort settle in shallow coastal waters, usually characterized by benthic vegetation, as early as February. With increasing water temperatures, juveniles grow rapidly to adolescents emigrating to offshore waters beginning in May, with the majority recruiting in June and July (Bishop 1988, Sideek et al. in press). Depending on spawning success and environmental conditions, a second recruitment in August or September from the late spring cohort may contribute to record commercial catches (Siddeek et al. in press). The success in development of the autumn cohort, however, remains uncertain and might largely depend on the environmental factors, and thus its contributions to the fishery is poorly known.

The shrimp fishery in Kuwait's waters has been regulated since 1981 by a fishing season closure in order to reduce the fishing pressure and allow the new recruits to grow to commercial size. Season opens on 1 September and continues until catches drop to less than 80-120 kg per industrial boat day, characteristically in February, March or April. Morgan (1989) found that spawning biomass below this level detrimentally affects the following season's recruitment. During the fishing season closure, the population changes only because of the natural mortality and recruitment. This provides us with an opportunity to estimate the natural mortality if information on the recruitment and population abundance is available during this period. In this study, we developed a variant of the catch-curve model (see Ricker 1975, p. 33) to estimate the natural mortality of the green tiger prawn in Kuwait's waters with research survey data collected during closed season.

### MATERIALS AND METHODS

#### *A Variant of the Catch-Curve Model*

Assuming the monthly natural mortality (month<sup>-1</sup>),  $M$ , is constant for the adult shrimp stock, and the fishing mortality caused by the research survey is negligible (thus the fishing mortality is

zero), then the population number of a cohort declines in an exponential way because of the natural death:

$$N_{ij} = N_{0j}e^{-Mt}$$

where  $N_{0j}$  is the initial population size of cohort  $j$  at the beginning of the closed season,  $N_{ij}$  is the population number of cohort  $j$  in month  $i$ ;  $t$  is the relative time scale indicating the length of the period that a cohort is exposed to natural mortality from time 0, i.e., the beginning of the closed season, to time  $t$ . Therefore,  $t$  is irrelevant to the actual age of the shrimp. Suppose there are  $J$  cohorts in the population which have the same natural mortality and the same probability of being captured by the sampling gear, then the total population number in month  $i$  can be calculated by:

$$N_i = \sum_{j=1}^J N_{ij} = \sum_{j=1}^J N_{0j}e^{-Mt} = e^{-Mt} \sum_{j=1}^J N_{0j} = N_0e^{-Mt}.$$

Assuming the catchability coefficient,  $q$ , is constant from month to month, then:

$$N_i = \frac{CPUE_i}{q} = \frac{CPUE_0}{q} e^{-Mt}$$

where  $CPUE_i$  and  $CPUE_0$  are catches in number per unit effort or relative abundances of the population, respectively, in month  $i$  and the beginning of the closed season, therefore,

$$CPUE_i = CPUE_0 e^{-Mt}. \quad (1)$$

The linear regression model of the logarithmic  $CPUE$  in month  $i$  on time  $t$  can be analyzed by using the method of least squares with a series of relative abundance data, i.e.,

$$\text{Log}(CPUE_i) = \beta_0 + \beta_1 t + e_i$$

where  $\beta_0 = \text{Log}(CPUE_0)$ ,  $\beta_1 = -M$ , and  $e_i$  is assumed to be independent random variable with mean zero and constant variance.  $\text{Log}$  denotes natural logarithm. Note that random measurement errors were present in the relative abundance,  $CPUE_i$ , obtained from the research survey; however, these errors were absorbed in the model error term,  $e_i$ , because the measurement errors were random and uncorrelated (Neter et al. 1990).

The bootstrap method, a general approach to testing the accuracy of an estimator (Efron and Tibshirani 1986), was applied to examine the estimation performance of the proposed method in this study.

### Research Vessel Survey

Monthly research surveys were conducted with a double-rigged industrial trawler leased from the United Fisheries of Kuwait during the closed shrimp season in 1993. Two-day monthly surveys from April through August (Table 1) sampled 10 to 17 stations from Kuwait Bay to Umm Al-Maradem Island in Kuwait's southern waters (Fig. 1). One tow at each station with two 25-m chain line width (between otter boards) trawls of 50 mm stretched mesh lasted for approximately 30 min in April, May and June and 15 min in July and August. Shorter tow duration reduced excessive catch volume of newly recruited fin-fish. All shrimp were sorted from both nets of each tow and weighed prior to examining all, or a 3 to 5 kg subsample, to determine species composition and size frequency. Carapace length was measured to the nearest 1 mm.

Based on shrimp species distributions and geographical considerations, Kuwait's waters were partitioned into 3 areas: Kuwait Bay which serves as nursery habitat and a staging area for the green tiger prawn, jinga shrimp, *Metapenaeus affinis* H. Milne Edwards 1837, and kiddi shrimp, *Parapenaeopsis stylifera* H. Milne Edwards 1837, and is protected from trawling year around; the middle area which supports the same species, but is subject to trawling during season; and the southern area, where landings are almost exclusively green tiger prawns (Fig. 1). Since this distribution pattern is relatively stable, and new recruits are more easily separated from the adult stock in the southern area, we used only the survey data from the southern area. By doing so, we assumed that the immigration of adult shrimp into the southern area equalled emigration of adult shrimp from the area. We use the terms shrimp and prawn interchangeably.

## RESULTS

### Separation of Adult and Recruitment Groups

During the closed shrimp season, the population declines because of natural death; however, there is also an increment in population size due to recruitment to the population during this period. Therefore, it is necessary to separate the adult groups from the recruitment groups before the natural mortality can be estimated. Only one mode was observed in April and May in the southern area and was considered of adult stock (carapace length  $\delta \geq 25$  mm and  $\text{♀} \geq 30$  mm). The recruitment stock started immigration into the southern area in June, and was not fully exploited by the fishing gear until July (Fig. 2). The modes of recruitment stock were clearly separated from those of the adults, and thus the relative abundance, or catch in number of adult shrimp per hour

TABLE 1.

Relative abundance, or catch in number (CPUE) of adult green tiger prawns per hour tow in Kuwait's southern waters.

Survey Dates	Relative Time Scale (Month)	Female			Male			Total	
		CL (mm)	CPUE (No./hr)	S.E.	CL (mm)	CPUE (No./hr)	S.E.	CPUE (No./hr)	S.E.
24-25 April	0	44.0	72.85	22.85	26.3	94.42	44.31	167.27	66.24
9-10 May	0.5	44.5	81.74	22.75	29.2	107.21	28.96	188.95	47.85
12-13 June	1.63	45.8	36.17	11.61	31.6	42.98	18.07	79.15	28.83
11-13 July	2.63	46.0	35.92	6.72	33.1	35.07	12.98	70.99	17.22
15-16 August	3.76	47.6	35.89	21.05	33.5	20.57	4.60	56.46	21.83

S.E. indicates the standard error of the relative abundance. CL indicates the mean carapace length of adult green tiger prawns.

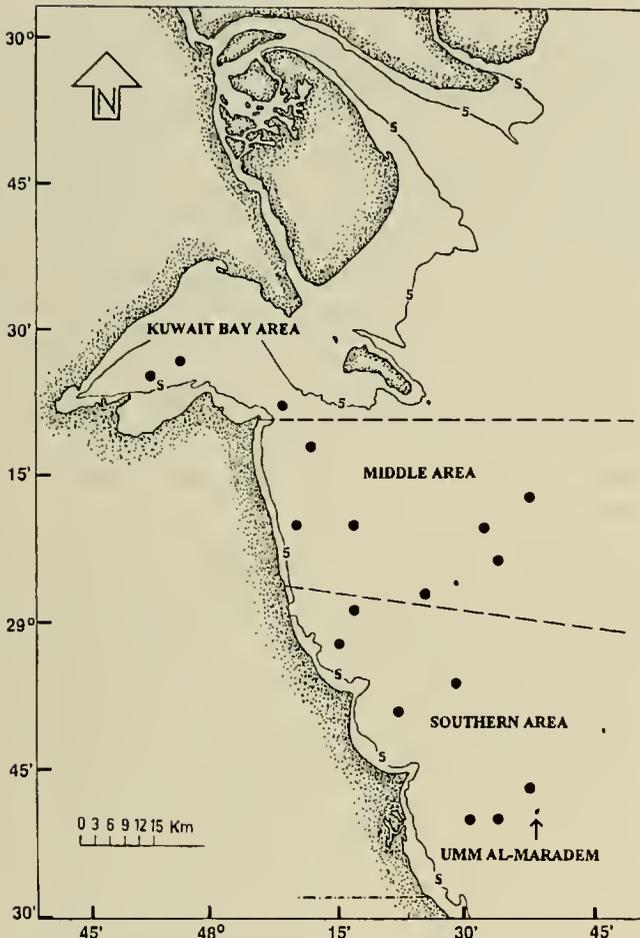


Figure 1. Trawl stations in Kuwait's waters from April through August, 1993. Dots indicate survey stations; broken lines are the boundaries between areas.

tow, from the southern area for each month were obtained (Table 1). The mean carapace lengths for the male and female adults stocks showed consistent increments from April through August (Table 1).

*Estimation of Natural Mortality Rate*

The natural mortality rates were estimated to be 0.23 and 0.44 month<sup>-1</sup>, respectively, for female and male shrimp by the proposed method (Table 2). The estimates were not significantly different ( $p > 0.05$ ) between the female and male shrimp. Therefore, the data for male and female were combined, and the natural mortality rate was estimated to be 0.33 month<sup>-1</sup> using the sexes combined data.

The confidence interval is a direct measure of accuracy of an estimate. The confidence interval can be obtained directly from the regression model if the errors,  $e_i$ , are assumed all from the same normal population,  $N(0, \sigma^2)$ . In this study, however, the nonparametric bootstrap method (Efron 1982), which does not require the normality assumption, was applied. 200 bootstrap estimates of natural mortality rates were obtained for male, female and sexes combined shrimp, respectively, by randomly resampling with replacement from the residuals of the fitted regression model. The confidence intervals for the estimated natural mortality rates (Ta-

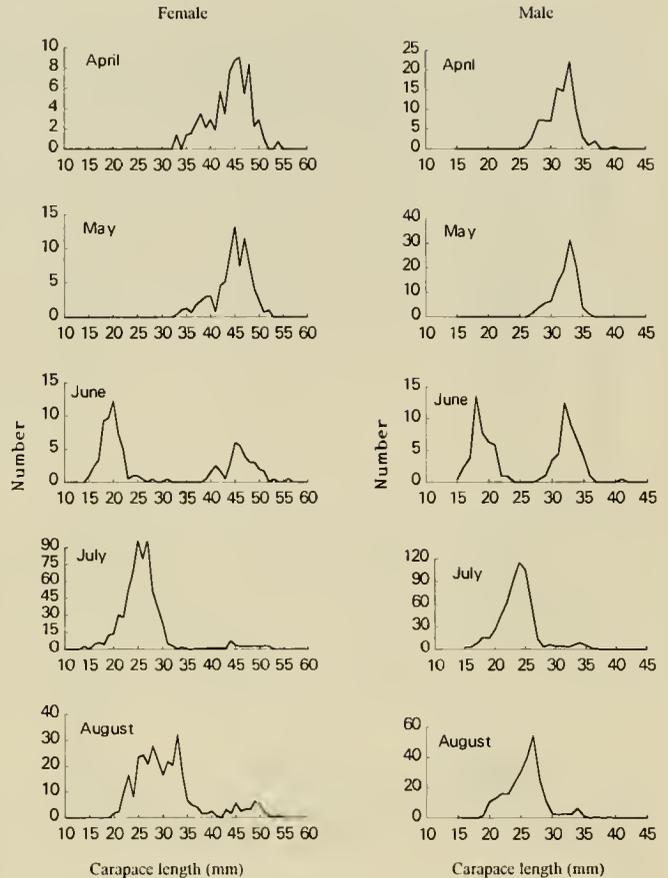


Figure 2. Length frequency of green tiger prawns in Kuwait's southern waters from April through August, 1993. Number scales change across months.

ble 2) were then constructed from these 200 bootstrap estimates by using the percentile method (Efron 1982, Hall 1992).

The estimation performance of the proposed method in this paper was examined with bootstrap simulation. The relative errors of 200 bootstrap estimates,  $M_b$ , comparing to the true value of the natural mortality rate (original estimate),  $M_o$ , were calculated by

$$\frac{M_b - M_o}{M_o} \times 100$$

and the relative error distributions presented by boxplots (Fig. 3).

TABLE 2.

Estimation of natural mortality rates of female and male green tiger prawns in Kuwait's waters with the proposed method.

Sex	Natural Mortality Rate (Month <sup>-1</sup> )	S.E.	r <sup>2</sup>	F	p
Female	0.23 (0.11–0.36)	0.08	0.73	7.93	0.067
Male	0.44 (0.35–0.53)	0.06	0.95	52.41	0.005
Combined	0.33 (0.23–0.43)	0.07	0.88	22.24	0.018

S.E. indicates the standard error by the regression model. r<sup>2</sup> is coefficient of determination. F represents calculated value from F test statistic, and p indicates the probability corresponding to the calculated F value. Values in parentheses are 95% percentile confidence intervals constructed by the bootstrap and percentile methods.

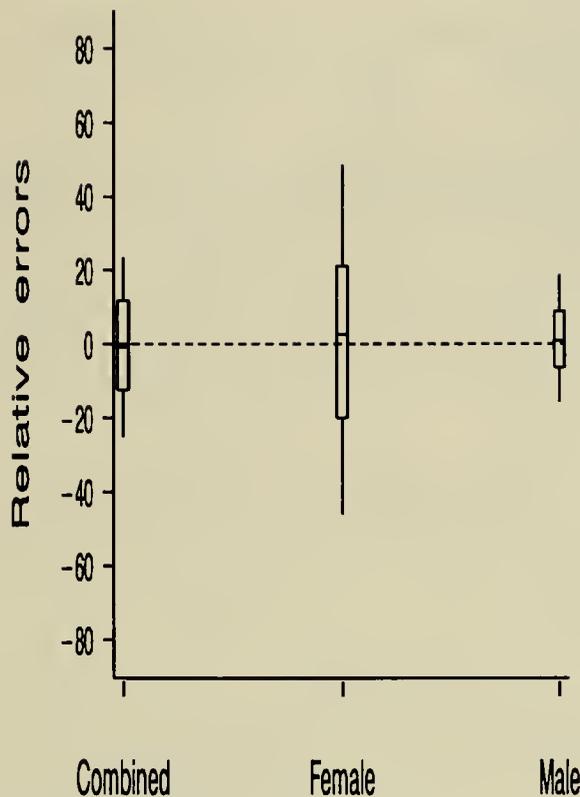


Figure 3. Boxplots of relative errors of estimates generated by bootstrap simulation compared to the original estimate of natural mortality rate.

The bottom and top edges of the box represent the 25th and 75th percentiles; whereas the bottom and top ends of the vertical line are the 5th and 95th percentiles. The medians (the 50th percentile) of 200 bootstrap estimates for female, male and sexes combined shrimp, shown as a bar in the box, are very close to the true value. Therefore, the estimation performance of the proposed method as an estimator for natural mortality rate is satisfactory.

#### DISCUSSION

##### *Survival of a Cohort with the Estimated Mortality*

Total mortality rates estimated by H. Mohammed (Kuwait Institute for Scientific Research 1994, unpublished data) using the

method of decline in CPUE over time with 1992/93 commercial fishery data were 0.50, 0.57 and 0.54 month<sup>-1</sup>, respectively, for green tiger prawn females, males and sexes combined. The cohorts exploited in this season were the same cohorts for which the natural mortality rates were estimated by the proposed method. Therefore, the natural mortality rate accounted for approximately 46, 77 and 61% of the total mortality, i.e., the fishing mortality rates were 0.27, 0.13 and 0.21 month<sup>-1</sup>, respectively, for females, males and sexes combined. The annual survival rates for female, male and sexes combined shrimp from September 1992 through August 1993 were therefore estimated to be 0.01, 0.002, 0.0044 yr<sup>-1</sup>, respectively. With such a low survival rate, a cohort would not survive through 2 successive fishing seasons. This might explain the short longevity of approximately 2 years for coastal penaeid shrimp stocks (Garcia and Le Reste 1981).

##### *Various Methods Used to Estimate Natural Mortality of Shrimp in Kuwait's Waters*

Different methods have been applied to estimate the natural mortality rate of green tiger prawns in Kuwait's waters (van Zalinge, see FAO 1982, Mathews et al. 1987, Siddeek et al. 1989, Siddeek 1991, Al-Hossaini 1993) with fairly different results (Table 3). The disparities in the estimated natural mortality for green tiger prawns by Pauly's regression model (Pauly 1980) were mainly due to the choice of growth parameters. Van Zalinge (see FAO 1982) and Al-Hossaini (1993) applied the infinite carapace length into Pauly's regression model; while Mathews et al. (1987) and Siddeek et al. (1989) used infinite total length converted from infinite carapace length by the regression equation of total length on carapace length. Two errors may be added to the estimate of natural mortality by Pauly's model when the infinite total length is converted from the infinite carapace length: 1) the predicted infinite total length by the regression equation of total length on carapace length tends to be seriously biased since the infinite carapace length usually lies out of the range of the carapace length data which are used to derive the regression equation; 2) parameter *K* corresponding to the infinite total length in the von Bertalanffy equation may be different from the *K* corresponding to the infinite carapace length. The validity of applying Pauly's regression method to shrimp stocks is a real concern because the data used by Pauly (1980) to develop the regression equation included only fish species, and the relation of natural mortality and growth param-

TABLE 3.  
Different estimates of natural mortality for green tiger prawns in Kuwait's waters.

Methods	Authors	Estimated Natural Mortality (yr <sup>-1</sup> )		
		Female	Male	Combined
Pauly's regression model	van Zalinge (see FAO 1982)	—	—	3.0
Pauly's regression model	Mathews et al. 1987	1.8	1.8	—
Pauly's regression model	Siddeek et al. 1989	2.4	2.3	—
Pauly's regression model	Al-Hossaini 1993	3.3	3.5	—
Length converted catch curve	Siddeek et al. 1989	—	1.8	—
Z vs fishing effort regression	Siddeek et al. 1989	1.8	1.9	—
CPUE ratio	Siddeek 1991	1.3–1.7	2.5–2.6	—
Cohort formula	Siddeek 1991	—	2.4	—
Modified Gulland's method	Siddeek 1991	1.7–2.0	2.0–2.3	—
The proposed method in present study		2.8 (1.32–4.3)	5.3 (4.2–6.4)	4.0 (2.8–5.2)

Values in parentheses are 95% percentile confidence intervals constructed by the bootstrap and percentile methods.

ters for shrimp might not follow this regression equation for fishes. However, if Pauly's regression is the only choice for estimating natural mortality of shrimp, the infinite carapace length might be preferred to the infinite total length.

The regression model of total mortality  $Z$  on fishing effort (Beverton and Holt 1957) was applied by Siddeek et al. (1989) to estimate the natural mortality. A serious problem for this method, however, is that the independent variable, i.e., fishing effort, is usually measured with errors, which violates the ordinary regression assumptions (Ricker 1975). Therefore, the estimators for regression parameters obtained by standard least squares are biased and lack consistency (Neter et al. 1990).

Siddeek et al. (1989) applied the length-converted catch curve (Gayaniilo et al. 1989) to the research survey data from May to July, 1987, during closed shrimp season. The sensitivity of the length-converted catch curve method for estimating mortality rates was investigated by Isaac (1990). She found that the estimate of mortality rate is positively related with von Bertalanffy growth parameters,  $L_{\infty}$  and  $K$ , and more sensitive to the bias in  $K$ . The effect of  $L_{\infty}$  and  $K$  on the estimate of mortality might be greater for shrimp than for fish since the converted age scale is in months; thus, a small variation in length might produce a big bias in estimated age. Moreover, when a deterministic growth equation is applied to convert a length scale into a time scale, some uncertainties are also being incorporated into the time scale which is the independent variable in the regression model, and thus the assumptions for the ordinary regression approach are violated.

Siddeek (1991) used the ratio of the CPUE data at the end of the fishing season ( $CPUE_1$ ) and the beginning of the next fishing season ( $CPUE_2$ ) to estimate the natural mortality during the closed shrimp season for a certain length group of adult shrimp. He selected a length group at the end of the fishing season and applied von Bertalanffy growth equation to calculate the length range of this group at the beginning of the next fishing season. Then the natural mortality rate for this period,  $M_p$ , can be calculated by  $M_p = \ln(CPUE_1/CPUE_2)$ . He noted that this estimator was very sensitive to the variation in the length intervals selected. Since only 2 points were considered in this estimator, i.e., only one straight line was defined from the two points, a small change in 1 of 2 points would significantly change the slope of the line, and thus change the estimate of natural mortality. The proposed method in this study overcomes this problem by considering a series of points during the closed season. In addition, the proposed method does not require a growth equation. This will eliminate the uncertainties caused by converting length to age. In the proposed method, the

independent variable of the regression model, i.e., the relative time scale, can be measured exactly according to the survey date, therefore, the regression model of relative abundance on the time can be appropriately applied to the data.

The proposed method in this study showed that the natural mortality rate for green tiger prawn females was lower than that for males, although the difference was not statistically significant ( $p > 0.05$ ). This finding is also supported by the results from the CPUE ratio method and the modified Gulland's method with tagging data by Siddeek (1991) and is consistent with the fact that adult female shrimp are bigger in size than adult males. Adult female shrimp are more vulnerable to capture by the trawl, and consequently, experience higher fishing mortality than males (0.27:0.13).

#### *Some Concerns in Application of the Proposed Method*

The precision in the estimated relative abundance (CPUE) will directly affect the estimate of the natural mortality rate. More sampling stations, i.e., bigger sample size, will reduce the variance for estimated CPUE, and thus increase the precision in the estimate of natural mortality. In this study, 5 to 7 stations were sampled in the southern area, and the variations in CPUE were fairly large (Table 1: range of CVs defined as S.E./CPUE from 18.7 to 58.7). Therefore, more sampling stations should reduce the variations.

Since the proposed method is based on the regression theory, the assumptions for the regression model must be followed in order to get a valid estimate, e.g., CPUEs by research survey for different cruises must be independent of each other. This would require a random sampling scheme for the survey, or if fixed stations were trawled, such as in this study, the shrimp in the survey area must be randomly distributed so that the CPUE for a station in one survey would be uncorrelated to that in the others.

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## GROWTH AND MORTALITY OF THE GIANT AFRICAN RIVER PRAWN *MACROBRACHIUM VÖLLENHOVENII* (HERKLOTS: CRUSTACEA, PALAEMONIDAE) IN THE LOBE RIVER, CAMEROON: A PRELIMINARY EVALUATION

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**ABSTRACT** A preliminary evaluation was carried out on growth data of the giant African River prawn, *Macrobrachium vollenhovenii*, in the river Lobe, Cameroon, collected between October 1990 and September 1991. Distribution of length indicated no definite spawning period within the year with total lengths falling in the range 4.5–13.1 cm. Estimates of growth parameters gave an asymptotic total length ( $TL_{\infty}$ ) of 16.41 cm, a curvative parameter ( $k$ ) of 3.19 year<sup>-1</sup>, a growth performance index ( $\phi$ ) of 2.93 and asymptotic carapace length ( $CL_{\infty}$ ) of 5.40 cm. The asymptotic body weight estimates ( $W_{\infty}$ ) ranged from 56.8 to 97.9 g over the different quarters of the year and averaged 60.97 g for overall data. There was indication of isometric growth. The values calculated for culture indices ( $CI = 4.66$  and  $CI' = 8.06$ ) showed that much potential lies in the use of *M. vollenhovenii* as a culturable species. Estimates of mortality parameters based on length frequency data were  $Z = 3.966 \text{ year}^{-1}$ ,  $M = 3.608 \text{ year}^{-1}$  and  $F = 0.258 \text{ year}^{-1}$ . The rate of exploitation ( $E = 0.07$ ) appears low.

**KEY WORDS:** Giant African River prawn, multiple spawning, growth parameters, growth pattern, mortality

### INTRODUCTION

Although the prawn, *Macrobrachium vollenhovenii* (Herklots), is common in Cameroonian fresh and brackish waters, little is known of its bioecology, and more particularly, its growth.

Several studies carried out on similar species of *macrobrachium* include those on the effects of different diets of *M. rosenbergii* (Manik 1976, Nelson et al. 1977, Boonyarat and New 1982, Millikin et al. 1980), on the salinity of *M. rosenbergii* (Smith et al. 1982), on water quality of *M. rosenbergii* (Summer and Evergole 1978, Cripps and Nakamura 1979, Menasveta 1982), on the quantification of energy income and investment of *M. rosenbergii* (Kumari and Pandian 1987), on induced spawning and laboratory rearing of *M. vollenhovenii* (Anetekhai and Fagade 1987), on the distribution of *M. vollenhovenii* (Hothius 1980, Powell 1982) and on the taxonomy and culture of *M. vollenhovenii* (Lackey and Nielsen 1980).

Some work on growth patterns and modelling of population dynamics of crustaceans is given in Garcia and Le Reste (1981), Jamieson and Bourne (1986) and Caddy (1987). These, with the review in Sparre et al. (1989), show that the average growth curve of a cohort of crustaceans does conform to the von Bertalanffy (1934) model. The presence of *M. vollenhovenii* in both the Barombi-Mbo crater lake and river in Kumba, Cameroon (9°22'E and 4°38'N) was reported in Trewavas et al. (1972). The commercial importance of this species is due to the high demand for it as food, the use of sun-dried smaller members as condiments for flavouring food (Sagua 1980) and its use as a foreign exchange earner. It is important to increase knowledge of the state and level of exploitation of *M. vollenhovenii* in the Lobe river.

The aim of this study is to give basic information on growth and mortality of *M. vollenhovenii*, with anticipation of more work

on its reproduction and ecology. This will aid in future decisions on the culture and rational management of this pelagic species.

### MATERIALS AND METHODS

The sampling site was the Lobe river about 8 km south of Kribi (9°56'E and 2°54.74'N). At the estuary of the river are falls which separate its fresh water from the salt water of the Atlantic Ocean.

Along the Lobe river are several landing sites dominated by indigenous artisanal fishermen who fish for prawns and other pelagic riverine fish species on a part-time basis. They use dug-out canoes of lengths ranging from 6 to 8 meters. These are hand paddled with between 2 and 3 paddles and 2 to 3 fishermen per fishing trip.

These fishermen use basket traps (4–9 cm mesh size) of length about 40–100 cm with diameter of 14.2–14.5 cm, similar in structure to those described by Trewavas et al. (1972) used in the fishing of Cichlids, *Macrobrachium* species and some freshwater catfishes in the lake Barombi-Mbo and river Kumba. The traps are designed so that when a prawn enters, it is trapped. They usually contain bait of either fresh ripe palm nuts, cassava or leaves. Setting usually takes 8 hours (2 am to 10 am) within the rocky shores of the river with suitable vegetative cover and defense territories. Such areas are suitable for the pelagic existence of *M. vollenhovenii*.

A total of 598 samples of prawn were collected from the fishermen twice a month, between October 1990 and September 1991. The total length (TL) and carapace length (CL) in centimeters were obtained by the use of a measuring board, while the total weights ( $w$ ) in grams were obtained by the use of a sensitive top loading Mettler balance (Model Sartorius GMBH Göttingen, type PT 600) with precision of 0.01 grams.

### Analysis of Data

#### Length-Frequency Distribution

The length-frequency distribution was analyzed by first pooling on a quarterly basis (the first quarter being October-December, 1990). This procedure is recommended by Pauly (1983) in cases where recruitment fluctuates. The computer package Length Frequency Distribution Analysis (LFDA) of Holden and Bravington (1992) was used for length-frequency data analysis, with the following methods used: SCLA (Shepherd's Length Composition Analysis devised by Shepherd, 1987) and ELEFAN (Electronics Length Composition Analysis devised by Pauly, 1987).

These methods estimate the parameters for the von Bertalanffy growth equation:

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

with parameters K (growth rate or curvature parameter),  $L_\infty$  (asymptotic length),  $t_0$  (time at which length equals zero). The package MIX developed by Macdonald and Pitcher (1979) was used in determining the age groups from the distribution of total lengths. Macdonald (1987) noted that this program remains an important application in the analysis of fisheries length-frequency data.

#### Growth Performance Index ( $\phi$ )

This was done by the application of the equation for  $\phi$  developed by Pauly and Munro (1984). It states that:

$$\phi = \text{Log}_{10}(k) + 2 \text{Log}_{10}(L_\infty)$$

with parameters as defined above. The growth performance index ( $\phi$ ) is an indication of the well-being of an aquatic species relative to its external milieu. Pauly and Munro (1984) assumed that whenever the same units are used, the quantity  $\phi$  is normally distributed within different populations of a given species.

Two culture indices (CI and CI') used in choosing fish for culture by Matthews and Samuel (1990, 1992) were used in the data analysis. They are defined as:

$$CI' = \phi' P$$

where  $\phi'$  is the growth performance index ( $\phi$ ) developed by Pauly and Munro (1984) using asymptotic body length,  $L_\infty$ , and:

$$CI = \phi P$$

where  $\phi$  is the growth performance index defined by Moreau et al. (1986) using asymptotic size,  $W_\infty$ , as follows:

$$\phi = \text{LOG}_{10} K + (2/3) \text{Log}_{10} W_\infty$$

P is the mean annual price (wholesale or retail) in US dollars per kilogram of fresh total weight, used as standard, and k is as defined above. CI determined using weight instead of length is of more interest to aquaculturists.

#### Growth Pattern

Scatter diagrams of the total length against carapace length and of logarithm (base 10) of weight against the logarithm (base 10) of total length were plotted. Least squares fit of the two linear regressions were derived, in the latter case giving estimates of a and b in the following equation, assuming  $\log_{10}(W)$  to be normally distributed:

$$W = a \text{ TL}^b$$

where

W = weight in grams,

TL = total length in centimeters

The growth patterns of this species was compared under the variable seasonal conditions, by forming the regression lines for each quarter separately.

#### Mortality Estimates

Two methods given in the computer package Length Frequency Distribution Analysis (LFDA) of Holden and Bravington (1992) were used in the estimation of the instantaneous rate of total mortality (Z) from the Beverton and Holt (1956) and Powell-Wetherall developed by Powell (1979) and extended by Wetherall et al. (1987). The instantaneous rate of natural mortality (M) was estimated from Pauly's (1980) empirical length growth equation using 28°C as mean water temperature (T). The equation states:

$$\begin{aligned} \text{Log}_{10}(M) = & -0.0066 - 0.279 \text{Log}_{10}(L_\infty) \\ & + 0.6543 \text{Log}_{10}(K) \\ & + 0.4634 \text{Log}_{10}(T) \end{aligned}$$

with parameters M,  $L_\infty$ , K and T as defined above. The instantaneous rate of fishing mortality (F) was calculated from the equation  $Z = F + M$  (Ricker 1975). This was followed by estimating the exploitation ratio (E), given as F/Z.

## RESULTS

#### Length-Frequency Distribution

The length-frequency study was made on all 598 specimens. The pooled quarterly distributions of length are given in Figure 1. The total length distribution ranged from 4.5 to 13.1 cm with a mean value of 8.31 cm while the carapace lengths ranged from 1.3 to 6.4 cm with a mean of 3.24 cm. The total length histograms showed several modes indicating that there was no definite spawning period within the year. The maximum total lengths, which fell in the 13–13.5 cm length range, were caught between January–March and July–September.

#### Growth Parameters Estimation

The best parameter estimates obtained using SLCA and ELEFAN methods are shown in Table 1. ELEFAN found 2 local maximums. The best estimate of asymptotic length obtained by ELEFAN method was close to that obtained using SLCA.

The growth of the population on an annual basis can be described using the von Bertalanffy equation:

$$L_t = 16.41\{1 - \exp[-3.19(t + 0.866)]\} \text{ cm TL}$$

estimated using SLCA. This equation can be used to describe the growth curve for *M. vollenhovenii*. The longevity ( $t_{\max}$ ) of *M. vollenhovenii* estimated from its maximum observed length ( $L_{\max} = 13.5$  cm TL) and equation  $t_{\max} = 3/K$  (Pauly 1983) approximated to 0.94 years. This suggests that *M. vollenhovenii* is a short-lived species.

#### Growth Performance Index ( $\phi$ )

By using the value of K = 3.19 and  $TL_\infty = 16.41$  obtained using SLCA in Pauly and Munro's (1984) equation, the growth performance index was calculated to be 2.93. By using a P value of US \$2.75/kg and  $\phi'$  of 2.93 for *M. vollenhovenii*, the value of

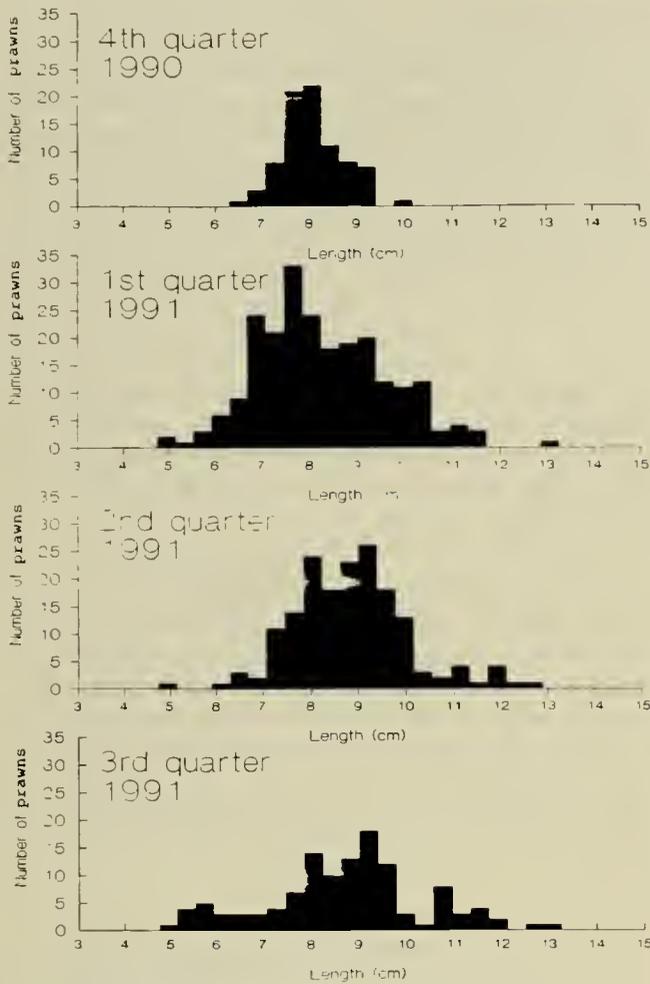


Figure 1. Histograms of quarterly length-frequency data for *M. vollenhovenii* formed by amalgamation of monthly data.

CI' was calculated as 8.06. An asymptotic weight,  $W_{\infty}$ , of 60.97 g and K of 3.19 from overall data gave  $\phi$  (Moreau et al. 1986) of 1.69 with a CI value of 4.66.

#### Growth Pattern

Application of the program MIX by MacDonald and Pitcher (1979) gave 3 normal components (Table 2) to the distribution of lengths, indicating the existence of 3 age groups in the *M. vollenhovenii* sampled.

A scatter plot of total length versus carapace length is shown in Figure 2 with each point identified by quarter and the 4 regression

TABLE 1.

Best growth parameter estimates obtained for *M. vollenhovenii* using SLCA and ELEFAN methods (Holden and Bravington 1992).

Method	Estimates			
	K	L (m)	T (years)	Score
SLCA	3.19	16.41	-0.866	33.33
ELEFAN	1.51	16.02	-0.450	0.361
	1.91	18.33	-0.290	0.255

TABLE 2.

Characteristics of different components of the distribution of lengths of *M. vollenhovenii*, determined using MIX (MacDonald and Pitcher 1979).

Components	Proportion	S.E.	Mean (cm)	S.E.	Sigma (cm)	S.E.
1	0.267	0.062	7.597	0.911	0.4992	0.101
2	0.609	0.079	8.404	0.108	1.6735	0.098
3	0.124	0.040	8.949	0.091	0.2682	0.090

Degrees of freedom = 13.

$\chi^2$  statistic = 6.506 ( $p = 0.926$ ).

lines for each quarter shown. The tests for common slope and intercepts were both highly significant (both  $p < 0.001$ ) and it is apparent the relationship for April to September data differs from the other 2 quarters.

The equations representing the relationship between carapace lengths and total lengths were as follows:

$$CL = -8.141 + 0.487 TL \quad (n = 598, r = 0.844)$$

(sexes combined)

$$CL = -2.892 + 0.466 TL \quad (n = 295, r = 0.871)$$

(females)

$$\text{and } CL = -4.460 + 0.398 TL \quad (n = 299, r = 0.815)$$

(males)

The sexes of 4 specimens of *M. vollenhovenii* could not be identified. Using  $TL = 16.41$  cm, the carapace length infinity ( $CL_{\infty}$ ) was estimated as 5.40 cm for sexes combined.

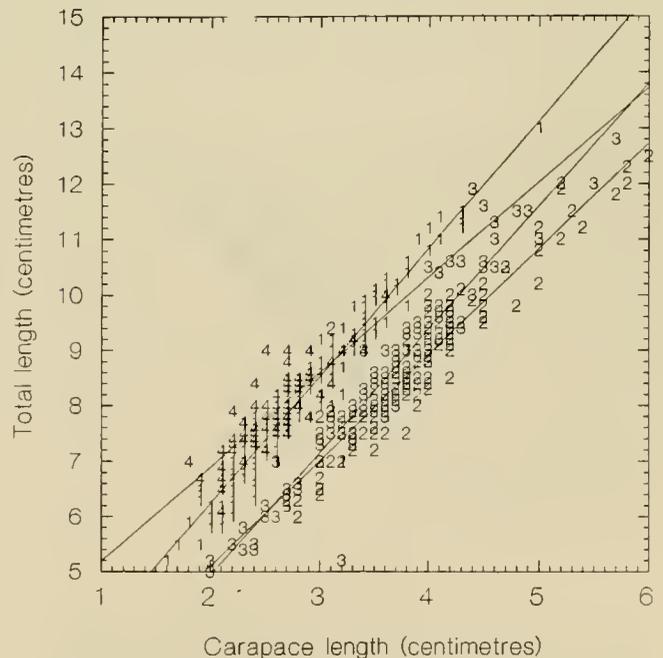


Figure 2. Scatter plot of total length versus carapace length, including fitted regression lines, for each quarter. Symbols and regression equations: 4 = Oct-Dec, 1990;  $TL = 3.48 + 1.71 CL$ , ( $n = 78$ ,  $r = 0.842$ ); 1 = Jan-Mar, 1991;  $TL = 1.59 + 2.31 CL$ , ( $n = 226$ ,  $r = 0.966$ ); 2 = Apr-Jun, 1991;  $TL = 1.21 + 1.93 CL$ , ( $n = 170$ ,  $r = 0.949$ ); 3 = Jul-Sep, 1991; and  $TL = 0.72 + 2.16 CL$ , ( $n = 120$ ,  $r = 0.963$ ).

Figure 3 shows the log weight-log length relationships. Again there were significant differences between quarters in relationships ( $p < 0.001$  for both intercepts and slopes) with April to September data again contrasting with October to March data, indicating a seasonal effect on the weight-length relationship of the species in the Lobe river. The overall log weight-log length relationship was:

$$\text{Log}_{10}(W) = -1.52 + 2.72 \text{Log}_{10}(\text{TL}),$$

$$(n = 598, r = 0.850)$$

Using the relationships for each quarter with  $\text{TL}_{\infty}$  estimated from all the data at 16.41 cm, the asymptotic body weights,  $W_{\infty}$ , were estimated to be 82.8, 97.9, 56.8, and 60.5 g for quarters 4, 1, 2, and 3 respectively. The exponents  $b$  were 2.70, 3.05, 3.00, and 3.08 respectively.

The equations for the relationship between log weight-Log length for both sexes were:

$$\text{Log}_{10}(W) = -4.736 + 2.920 \text{Log}_{10}(\text{TL}) \quad (n = 295,$$

$$r = 0.915) \text{ (females)}$$

$$\text{Log}_{10}(W) = -4.419 + 2.876 \text{Log}_{10}(\text{TL}) \quad (n = 299,$$

$$r = 0.953) \text{ (males)}$$

These gave exponents  $b$  to be 2.920 and 2.876 for the females and males respectively.

#### Mortality Estimates

Estimates of the von Bertalanffy growth parameters ( $L_{\infty} = 16.41$  cm TL,  $K = 3.19 \text{ year}^{-1}$  and  $T_0 = -0.866$ ) obtained using length frequency data were used to estimate total mortality rate ( $Z$ ). The estimates were  $2.85 (\pm 0.426 \text{ SD}) \text{ year}^{-1}$  (Beverton and Holt) and  $3.966 (\pm 2.54 \text{ SD}) \text{ year}^{-1}$  (Powell-Wetherall). No

significant estimate was obtainable for  $Z$  using the age-slice method.

An annual mean water temperature of  $28^{\circ}\text{C}$  was observed during this study. Using this value, the multiple regression equation of Pauly (1980) gave instantaneous rate of natural mortality of  $3.61 \text{ year}^{-1}$  with  $Z = 3.966 \text{ year}^{-1}$  from Powell-Wetherall. This gives the instantaneous rate of fishing mortality ( $F$ ) as  $0.258 \text{ year}^{-1}$ . The exploitation ratio ( $E = F/Z$ ) is 0.07 indicating a very low fishing intensity when compared to the optimum rate of 0.5 suggested for exploited stocks (Gulland 1971, Pauly 1983).

#### DISCUSSION

No modes were found in the pooled quarterly distributions of length and therefore, the conclusion was reached that, there was no definite spawning period within the year. More study involving ovary staging and quantification of presence or absence of berried females during the year may be necessary before definite conclusions can be drawn relative to spawning periodicity.

The observed maximum size (13.1 cm) obtained in this study was lower than the estimated asymptotic length (16.41 cm) and the value of 18.7 cm obtained by Holthius (1980) and Powell (1982). Both authors mentioned that *M. vollenhovenii* is the largest growing prawn in West Africa. There is therefore a need to carry out some management decisions such as controlling the mesh size of the basket traps used or operating closed seasons when fishing will not take place. These will enable the species to attain higher lengths and weights. Improvement on the maximum lengths and weights obtained in the wild can be attained by intensive culture which will result in improvement of the growth performance index.

An estimated longevity of 0.94 years for *M. vollenhovenii* shows it to be short-lived and hence growth performance may indicate suitability for culture. Values of the growth performance index ( $\phi$ ) are used to compare stocks of the same species. At present no other published values for *M. vollenhovenii* are known, for comparison with this study's estimate of 2.93. However, it is higher than other estimates ranging between 2.64 and 2.71 in some tropical invertebrate shrimps (*Penaeus merguensis*, *P. indicus*, *P. subtilis*, *Metapenaeus affinis* and *M. palmensis*) studied in Venema et al. (1988). Hence, freshwater *M. vollenhovenii* has shown a better growth performance than certain in the marine/estuarine environment.

The values of  $CI'$  of 8.06 and 4.66 fall within the ranges of 5.71 to 20.5 and 1.84 to 12.41 respectively indicated in Matthews and Samuel (1992) of some commercially important populations (*Acanthopagrus cuvieri*,  $CI = 12.41$ ,  $CI' = 20.5$ ; *Parapanaeopsis stylifera*,  $CI = 1.84$ ,  $CI' = 5.71$  and *Penaeus simusulcatus*,  $CI = 3.95$ ,  $CI' = 8.61$ ) of Kuwait. Matthews and Samuel (1990) noted that the culture potential from growth rate ( $\phi$ ) was only one of several inputs relevant for choosing fish for culture. Other factors used to determine culture potential besides growth rate were; the value of the fish and its popularity, i.e., its appeal to consumers as well as biological characteristics such as tendency to be cannibalistic or sensitivity to handling. The popular taste and general appeal can only be estimated by scrutinizing fish prices while biological characteristics can be determined experimentally. This justification shows that much potential lies in the use of *M. vollenhovenii* as a culturable species. However, more justification still has to be made from comparative studies with similar prawn and shrimp species of the same environment.

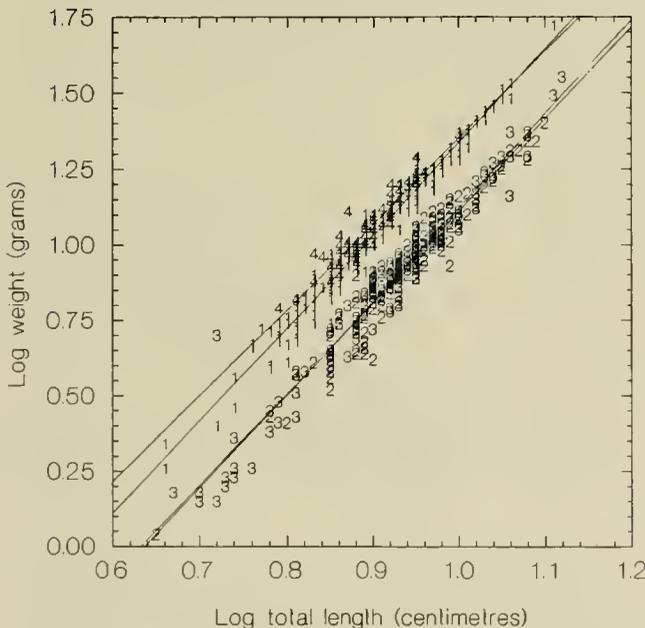


Figure 3. Scatter plot of log total versus log weight, including fitted regression, lines, for each quarter. Symbols and regression equations: 4 = Oct-Dec, 1990;  $\text{Log}_{10}(W) = -1.36 + 2.70 \text{Log}_{10}(\text{TL})$ , ( $n = 82$ ,  $r = 0.933$ ); 1 = Jan-Mar, 1991;  $\text{Log}_{10}(W) = -1.72 + 3.05 \text{Log}_{10}(\text{TL})$ , ( $n = 226$ ,  $r = 0.986$ ); 2 = Apr-Jun, 1991;  $\text{Log}_{10}(W) = -1.89 + 3.00 \text{Log}_{10}(\text{TL})$ ; ( $n = 170$ ,  $r = 0.965$ ); 3 = Jul-Sep, 1991; and  $\text{Log}_{10}(W) = -1.96 + 3.08 \text{Log}_{10}(\text{TL})$ , ( $n = 120$ ,  $r = 0.973$ ).

The differences between quarters in growth patterns shown in the total length—carapace length and low weight—log total length relationships in *M. vollenhovenii* is attributed to seasonal differences. The quarters of the year correspond to a minor rainy season (first quarter), major dry season (second quarter), minor rainy season (third quarter) and major rainy season (fourth quarter). These seasons were indicated in studies off Cameroon by Youmbi et al. (1991). Growth pattern from asymptotic weights showed faster growth within the major dry and raining seasons with slower growth within the minor dry and rainy seasons. The faster growth must have been due to higher productivity due to the presence of adequate sunlight and availability of nutrients in the river from flooding. Evidence for 3 age groups within the population of *M. vollenhovenii* sampled was quite significant ( $p = 0.926$ ) as the value of  $\chi^3$  obtained was low compared to values obtained in standard tables for 13 degrees of freedom.

All the measures taken to improve the growth performance index and indications from the growth pattern studied should enable supply to match demand of *M. vollenhovenii* as food and condiments for flavoring.

The exponents  $b$  obtained for the fourth quarter was below 3, those for the first and third quarter were above 3 while that for the second quarter was equal to 3. Those for different sexes were below 3. Tesh (1968) noted that when this exponent is close to 3, it indicates isometric growth when the specific gravity remains constant, but if well below or above 3, then it shows allometric growth. The above values apart therefore suggest isometric growth. The value of  $b = 2.72$  for the overall length weight relationship showed allometric growth. The differences in values

of  $b$  for the different quarters and sexes were an indication of sexual dimorphism in *M. vollenhovenii* with the 2 groups representing males and females.

In the Lobe river, mortality of *M. vollenhovenii* is largely due to fishing activities and predation from birds and reptiles. The instantaneous rate of natural mortality of  $3.61 \text{ year}^{-1}$  was high and probably resulted from the presence of piscivorous fishes and adverse environmental conditions. The low instantaneous rate of fishing mortality of  $0.258 \text{ year}^{-1}$  was due to the indigeneous fishermen along the Lobe carrying out fishing on a part-time basis. However, there is a high demand for *M. vollenhovenii* by the population of the touristic town of Kribi and its environs which can be met by increased fishing and introduction of intensive culture.

The exploitation ratio of 0.07 in the Lobe river is an indication of under-exploitation. This value does not exceed the optimum rate of 0.5 (Gulland 1971, Pauly 1983) estimated to give the highest yield per recruit for the population. This suggests an active exploitation of *M. vollenhovenii* with management measures being taken not to exceed this highest yield.

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## INTERACTIVE EFFECTS OF POLYCULTURE, FEEDING RATE, AND STOCKING DENSITY ON GROWTH OF JUVENILE SHELLFISH

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**ABSTRACT** Growth rates were evaluated for juvenile red abalone *Haliotis rufescens* (Swainson), mussels *Mytilus californianus* (Conrad), and spot prawns *Pandalus platyceros* (Brandt) cultured in monocultures and polycultures at 3 feeding rates. Two polyculture stocking densities were employed, one in which each species was at the same density as in monoculture, for a combined density of 4.6 g shellfish per liter, and another in which the combined density was the same as for a single species in monoculture (approximately 2.3 g/L). All culture groups were fed at 3 levels: unfiltered seawater only, and unfiltered seawater plus 2 different quantities of benthic diatoms (0.25 or 0.50 g dry weight/week), phytoplankton (10 or 50 × 10<sup>3</sup> cells/mL), and diced shrimp (3 or 10% of prawn weight per day). Feeding rate and culture treatment produced a significant interactive effect on shellfish growth (ANOVA *p* < 0.001). Abalone growth was significantly greater in monoculture than in either the lower or higher density polyculture (mean individual weight increase = 0.48, 0.33 and 0.28 g, respectively). Likewise, prawn growth was significantly greater in monoculture than in either polyculture (0.72, 0.44 and 0.41, respectively). Growth increased significantly with amount of food supplied: mean abalone weight increased 0.15 g in the lowest feeding treatment vs 0.60 g in the highest, and mean prawn weight increased 0.21 g in the lowest feeding treatment vs 0.88 g in the highest. Mussel growth (0.04 g) was restricted by food availability at all feeding rates, and was not significantly affected by any experimental manipulations. Despite expected differences in feeding habits among the 3 species, abalone were found to be capable of ingesting and assimilating diced shrimp that was intended as food for prawns in the polyculture system. Sequential polyculture is proposed as an alternative to combined culture for pilot-scale applications.

**KEY WORDS:** Red abalone *Haliotis rufescens*, spot prawn *Pandalus platyceros*, California mussel *Mytilus californianus*, hatchery, polyculture

### INTRODUCTION

Polyculture techniques have been used to increase production of fish in freshwater ponds for centuries (Bardach et al. 1972). The polyculture concept originated in ancient China, where a number of carp species were stocked together in earthen ponds to exploit the variety of available food and habitat (Chang 1987, Milstein 1992). More recently, several marine shellfish species have been grown in polycultures, augmenting harvests through wider use of available food and space, while minimizing the negative effects of species-specific exometabolites (Konstantinov and Yakovchuk 1993). Penaeid shrimp have been polycultured with milkfish, pompano, tilapia, and rabbitfish (Gundermann and Popper 1977, Tatum and Trimble 1979, Eldani and Primavera 1981). Pandalid shrimp have been polycultured with salmon (Rensel and Prentice 1979). Various bivalve species have been cultured with fish or shrimp to improve total yields and reduce waste stream particulate loads (Maguire et al. 1981, Shpigel et al. 1993, Jakob et al. 1993, Jones and Iwama 1991). Polyculture systems using oysters, mussels, clams, and abalone have been evaluated in conjunction with municipal waste water treatment (Tenore 1976, Mann and Ryther 1977).

The red abalone *Haliotis rufescens*, the mussel *Mytilus californianus*, and the spot prawn *Pandalus platyceros* have been cultured in commercial and pilot programs on the west coast of

North America (Hahn 1989, Skidmore and Chew 1985, Rensel and Prentice 1980). The 3 species have similar temperature and salinity tolerances, and their use of different foods and habitats suggest their potential for polyculture. Juvenile abalone graze benthic microalgae that readily colonize illuminated substrates in culture systems (Ebert and Houk 1984). Mussels are most efficiently cultured in the water column where they filter suspended particles and phytoplankton (Chew 1984, Skidmore and Chew 1985). Spot prawns are mobile omnivores (Wickens 1972, Weinberg 1981) whose diet includes fouling organisms and dead individuals of other cultured species (Rensel and Prentice 1979, 1980). A combination of these species in polyculture may improve exploitation of available food and habitat space in hatchery culture systems. Abalone occupying container walls could graze algal films, while mussels suspended in the water column filter particulate carbon, and prawns roam throughout, feeding on fouling organisms and moribund molluscs to supplement their diet. Higher overall densities could be achieved without a concomitant increase in species-specific metabolite concentrations.

We investigated the interactions among juveniles of these 3 species on an experimental scale to determine their suitability for hatchery polyculture. The 3 species were grown together and separately to determine how polyculture, feeding rate, and stocking density affected shellfish growth rate.

## MATERIALS AND METHODS

### Facilities

All experiments were conducted from July to November at the Marine Culture Laboratory (California Department of Fish and Game), located at Granite Canyon, 30 km south of Monterey, CA. Organisms were cultured in natural seawater pumped directly from well-mixed coastal waters adjacent to the laboratory (Ebert et al. 1974). Water temperatures ranged from 12° to 15°C and salinity ranged from 33 to 35‰ during the course of the experiments.

### Experimental Design

Culture type, feeding rate, and stocking density were manipulated; growth in length and weight of abalone, mussels and prawns was measured. Treatments consisted of monocultures of each species, and polycultures at 2 densities. In one polyculture, each species was stocked at the same density as in monoculture, so the combined density of the 3 species (4.6 g shellfish per liter) was higher than in any of the monocultures. In the second polyculture treatment, larger containers were used so that the combined density of all species was similar to that of a single species in monoculture (approximately 2.3 g/L). Stocking densities were 10 juvenile abalone per container (2.0 g/L), 20 juvenile mussels per container (2.2 g/L), and 4 post-larval prawns per container (0.43 g/L). Each polyculture contained 34 animals. The number of animals remained constant throughout the 100 day experiment, as dead individuals were replaced at monthly intervals by new individuals of similar size. (Overall mortality rate was 3.5%). Replacement animals were drawn from a pool of cohorts kept under culture conditions similar to those at the intermediate feeding rate. Replacement prawns were found to grow at rates similar to the mean for their respective treatment (unpublished data). The mortality rate for both abalone and mussels was approximately 1%; thus replacement was assumed to have a negligible effect on the experimental results.

In successful polyculture systems, trophic diversity allows greater production from the variety of available food items. In this experiment, food quantity was manipulated to examine the interaction between food availability and species composition. Monocultures and polycultures at both densities were fed at 3 rates, described below. The combination of 5 culture treatments (polycultures at 2 densities, and a monoculture for each of the 3 species) and 3 feeding rates was replicated 3 times, for a total of 45 experimental containers.

### Culture System

All culture containers were arranged in a stratified random pattern in a single large water bath that maintained even temperatures for all treatments (12° to 15°C). Each container received continuously flowing unfiltered natural seawater at an average rate of  $250 \pm 90$  mL/min (sd). Analysis of variance revealed no significant differences in water flows between treatments. All containers were illuminated by sunlight from a south-facing window, diffused by 2 horizontal layers of 1 mm mesh nylon screen covering the entire system.

Culture containers were 2-liter polyethylene food containers with 1,875 mL of water volume and 1,600 cm<sup>2</sup> of wet surface area (including internal structures, see below). The larger (lower density) polyculture containers were 4-liter polyethylene food con-

tainers with 3,700 mL of water volume and 2,300 cm<sup>2</sup> of wet surface area. Average water turnover time was 7.3 min in the smaller containers and 14.5 min in the larger containers. The containers were designed to provide variety of habitat, maximum use of 3-dimensional space, and access to existing food by each species. An open inner compartment confined the mussels on a small submerged screen through which all incoming water was forced to pass. This allowed the suspension feeders immediate access to incoming particulate material. Abalone and prawns roamed freely throughout the aquarium, which was divided into 4 sections by 2 diatom-covered plates that formed a free-standing cross 5 cm high (described below).

### Shellfish Source and Handling

Red abalone, *H. rufescens*, were spawned at the laboratory and cultured for 240 days prior to the experiment, using techniques described by Ebert and Houk (1984). Initial abalone mean length and weight ( $\pm$ coefficient of variation, CV) were  $14.9 \text{ mm} \pm 2\%$  and  $0.37 \text{ g} \pm 5\%$ . This size was chosen after preliminary experiments with spot prawn predation.

Mussels, *M. californianus*, were collected from the rocky intertidal near the laboratory 1 week prior to the start of the experiment. Initial mussel mean length and weight ( $\pm$ CV) were  $11.2 \text{ mm} \pm 2\%$  and  $0.21 \text{ g} \pm 3\%$ .

Spot prawn *P. platyceros* post-larvae were full siblings hatched in the laboratory from a single gravid female 97 days prior to the beginning of the experiment. Prawns grew from zoea to post-larvae on a diet that progressed from *Artemia* nauplii to adult *Artemia* to diced meat of bait shrimp and squid. Initial prawn mean length and weight ( $\pm$ CV) were  $14.1 \text{ mm} \pm 6\%$  and  $0.20 \text{ g} \pm 15.4\%$ .

All prawn length measurements were carapace length from the tip of the rostrum to the posterior mid-dorsal edge of the carapace. Abalone and mussel lengths were longest shell dimension. All were measured using Vernier calipers accurate to 0.1 mm. All weight measurements for all species were taken as live blotted wet weight on a laboratory balance accurate to 0.01 g. Regression coefficients for wet to dry weight were  $\geq 0.99$  for all species ( $n = 10$  per species).

Shellfish densities were similar to those used in other experimental studies. Monocultures and polycultures held 10 abalone, 20 mussels, and/or 4 prawns. The density of 5.3 abalone per liter was within the range of 3.3 to 11 per liter for similar sized abalone stocked into hatchery growout tanks by Ebert and Houk (1984). Mussel density of 10 per liter was half that used for similar sized mussels in feeding experiments by Fidalgo et al. (1994). The 400 cm<sup>2</sup> of wet surface area available per prawn in this study was somewhat larger than the 225 cm<sup>2</sup> for slightly younger *P. platyceros* in previous feeding experiments (Kelly et al. 1977). Intraspecific density was lower in the larger polyculture containers, as described above.

### Food and Feeding Rate

One suitable food was chosen for each of the 3 shellfish species: benthic diatoms for the abalone, cultured phytoplankton (*Isochrysis galbana*) for mussels, and diced bait shrimp for prawns. To maintain equality in the amount and type of food presented to both the polyculture and monoculture treatments, all

culture containers at a given feeding level were given the same amount of all 3 food types. There were 3 feeding levels. Level I received nothing but flowing unfiltered natural seawater. Level II received unfiltered seawater and known additions of benthic diatoms (0.25 g dry weight per week), phytoplankton (10,000 cells/mL 9 times per week), and diced shrimp (3% of prawn wet weight per day). Level III received unfiltered seawater and 0.50 g (dry weight) diatoms per week, 50,000 phytoplankton cells/mL 9 times per week, and diced shrimp (10% of prawn wet weight per day).

Benthic diatoms were grown on polystyrene plates in a separate tank. The tank was inoculated weekly with a slurry of benthic diatoms passed through a 5  $\mu\text{m}$  bag filter. Two of the rectangular plates, notched in the center, were fit together to form a free standing cross extending into the corners of the culture container. These provided 400  $\text{cm}^2$  of grazing surface that contained 0.25 g dry weight of organic growth, at least 50% of which was composed of chain-forming and solitary benthic diatoms, as observed microscopically. Treatments in food level II received 1 set of diatom-covered plates per week, and treatments in level III received 2 sets of plates per week. Dry weight of diatoms varied among plates by 21% (CV,  $n = 10$ ).

The specific composition of benthic diatoms was not determined, nor was there any measurement made of the type or quantity of particulate material suspended in the influent natural seawater. However, all experimental treatments received benthic diatoms and suspended particulate matter from the same sources, and the specific composition was expected to have minimal effect on any observed differences in growth rate between treatments. Local coastal waters from which seawater was drawn were generally clear, but well mixed and nutrient rich, supporting kelp forests and local beds of *M. californianus*. Seawater particulate loads were sufficient to allow 3 to 4 g (dry weight) of attached fouling material to accumulate over the course of the experiment in containers that were not actively grazed (generally mussel monocultures). Containers were rinsed each month during growth measurements to remove loose detritus. There was little substantial buildup of detrital material in any containers, due to relatively high water flow and the location of effluent ports at the bottom of each tank.

Phytoplankton (*I. galbana*) was cultured in 20 L carboys and harvested near the peak of the growth curve (after 7 to 10 days). Phytoplankton was delivered to culture containers 3 times per day, 3 days per week, by shutting off the seawater flow, adding measured volumes of phytoplankton, and leaving the containers static for 1 hour to allow clearance of the suspensions. Treatments in level II received enough phytoplankton to achieve a concentration of 10,000 cells/mL in the culture container. Treatments in level III received enough phytoplankton to achieve a concentration of 50,000 cells/mL in the culture container. Feeding rate was based on reported mussel filtration rate declines at concentrations greater than 50,000 cells/mL (Schulte 1975). Discontinuous feeding was used for logistical reasons, supported by reported success with discontinuous feeding of bivalves (Langton and McKay 1976). The combination of natural particulates in the unfiltered seawater and the regular additions of phytoplankton was expected to be sufficient for bivalve growth.

Food provided for prawns was bait shrimp abdominal tissue, cut into pieces averaging 0.05 g each. Treatments in level II received amounts equal to 3% of prawn weight per day, and treatments in level III received amounts equal to 10% of prawn weight per day. Food was delivered in equal portions 3 times per week.

#### Data Analysis

To determine shellfish growth rates, the mean initial individual shell length and live weight was subtracted from final values for each replicate. Mean growth in length and weight per individual per replicate were then compared using two-factor analysis of variance (ANOVA), with culture type and feeding rate as the 2 factors. A 3 by 3 two-factor analysis of variance was performed for each species. If a significant difference was detected between treatments, a Student-Newman-Keuls multiple comparison test was used to resolve differences at an alpha level of 0.05 (Zar 1974).

#### Food Utilization Experiments

To investigate possible competition for food between prawns and abalone, the following experiment was conducted. Two abalone were placed in each of 6 isolated containers with flowing 1  $\mu\text{m}$ -filtered seawater. Three of the containers were given no food, the other 3 were fed equal quantities of diced shrimp, the food intended for prawns in the polyculture experiment. Similarly, one prawn was put into each of 6 separate containers; 3 were starved, and 3 were given diatom covered plates, the food provided for abalone in the polyculture. After 33 days, growth and survival were measured in each replicate of each treatment and the results were compared using a t-test with an alpha level of 0.05 (Zar 1974).

While the reverse experiment was not performed, satisfactory growth has been observed for abalone fed similar quantities of diatoms (Ebert and Houk 1984), and prawn growth rates similar to those observed in this study's monocultures have been reported for optimal diced food diets (Kelly et al. 1977).

## RESULTS

Manipulation of both culture type and feeding rate resulted in significant differences in the growth of both prawns and abalones. Mussel growth appeared to be food limited and was not significantly affected by changes in either factor.

Abalone grew at significantly higher rates in monoculture than in polycultures (ANOVA,  $p < .001$ ; Table 1). Polyculture density significantly affected growth in abalone weight, but not length (SNK,  $p = .05$ ; Fig. 1). Prawns also grew faster in monoculture than in polycultures (ANOVA,  $p < .001$ ; Table 1). Polyculture density did not significantly affect prawn growth in length or weight (SNK,  $p < .05$ ; Fig. 2). Feeding rate significantly affected growth of both prawns and abalone, with significant differences between all feeding levels (ANOVA,  $p < .001$ , SNK  $< .05$ ; Table 1). Abalone in treatment level I (seawater only) were unaffected by culture type (ANOVA,  $p > .25$ ), whereas prawns in treatment level I were significantly affected by culture type, but not density (ANOVA,  $p < .005$ , SNK,  $p = .05$ ).

Total growth of all species combined was significantly greater in the low density polyculture relative to the higher density polyculture or any of the monocultures (ANOVA,  $p < .001$ ; Table 2; Fig. 3). There was no significant difference between the total combined growth of the higher density polyculture and the growth of abalone in monoculture (SNK,  $p < .05$ ). Total growth in prawn monoculture was significantly less than combined growth in the higher density polyculture, and growth of mussels was significantly less than that (SNK,  $p < .05$ ; Table 2). Combined growth

TABLE 1.

Increase in length and weight for juveniles of 3 shellfish species over a 100 day experiment.

Food Quantity Group	Monoculture	Polyculture	Large Polyculture	Mean
<b>A. Abalone growth in length (mm).</b>				
I	2.4 + 1.0	1.6 + 0.2	2.5 + 0.7	2.2
II	4.5 + 0.4	2.8 + 0.4	3.7 + 0.3	3.7
III	6.8 + 0.0	5.0 + 0.5	5.5 ± 0.5	5.8
Mean	4.6	3.1	3.9	3.9
<b>B. Abalone growth in weight (g).</b>				
I	0.19 + 0.13	0.08 + 0.01	0.18 + 0.07	0.15
II	0.46 + 0.03	0.23 + 0.03	0.31 + 0.02	0.33
III	0.79 + 0.05	0.53 + 0.06	0.49 + 0.05	0.60
Mean	0.48	0.28	0.33	0.36
<b>C. Mussel growth in length (mm).</b>				
I	0.6 + 0.6	0.6 + 0.1	0.5 + 0.7	0.6
II	0.3 + 0.2	0.5 + 0.4	0.5 + 0.2	0.4
III	1.2 + 0.4	0.9 + 0.4	0.4 + 0.3	0.8
Mean	0.7	0.7	0.5	0.6
<b>D. Mussel growth in weight (g).</b>				
I	0.04 + 0.03	0.02 + 0.01	0.03 + 0.04	0.03
II	0.03 + 0.02	0.03 + 0.03	0.04 + 0.02	0.03
III	0.08 + 0.03	0.04 + 0.03	0.02 + 0.02	0.05
Mean	0.05	0.03	0.03	0.04
<b>E. Prawn growth in length (mm).</b>				
I	7.3 + 0.6	2.0 + 1.7	3.3 + 0.6	4.2
II	10.0 + 2.0	7.3 + 1.5	6.0 + 1.0	7.8
III	12.7 + 0.6	11.0 + 1.0	11.0 + 1.0	11.6
Mean	10.0	6.8	6.8	7.9
<b>F. Prawn growth in weight (g).</b>				
I	0.38 + 0.03	0.10 + 0.08	0.16 + 0.04	0.21
II	0.71 + 0.16	0.37 + 0.12	0.32 + 0.05	0.47
III	1.06 + 0.05	0.76 + 0.10	0.84 + 0.07	0.88
Mean	0.72	0.41	0.44	0.52

Values are mean ± standard deviation for increases in length (in mm) or weight (in grams) in 3 culture treatments at 3 feeding rates.

was affected by feeding rate in both polycultures, with significant differences between all feeding levels (SNK,  $p < .05$ ; Table 2).

There was a significant interactive effect between culture type and feeding rate for abalone growth in weight and for total pro-

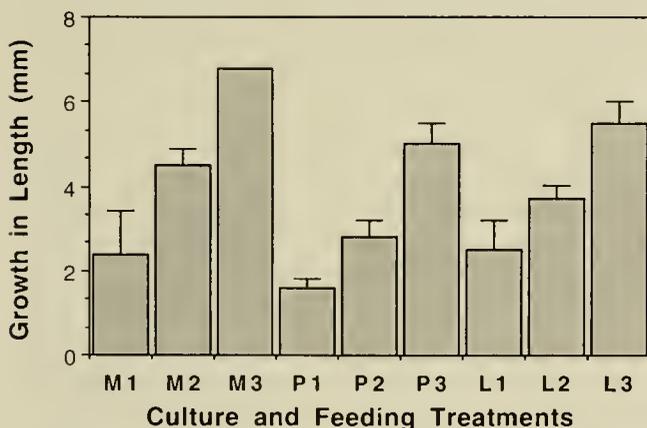


Figure 1a. Growth in length (mm) of juvenile abalone in monoculture (M), polyculture (P), and larger lower density polyculture (L) at 3 feeding rates (1, 2 and 3). Columns indicate mean increase in length ( $\pm$ standard deviation,  $n = 3$ ).

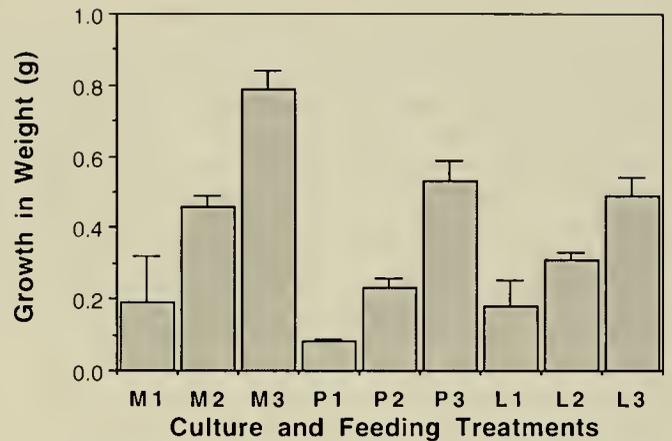


Figure 1b. Growth in weight (g) of juvenile abalone in monoculture (M), polyculture (P), and larger lower density polyculture (L) at 3 feeding rates (1, 2 and 3). Columns indicate mean increase in weight ( $\pm$ standard deviation,  $n = 3$ ).

duction of all species combined (ANOVA,  $p < .05$  and  $< .001$ , respectively).

Mortality rate was 1% for both abalone and mussels, and 18% for prawns over the 100 day experiment. Neither culture type nor feeding rate had any significant effect on mortality of any species (ANOVA,  $p > .25$ ).

There were no significant differences in growth or survival between prawns fed only diatoms and prawns that were starved. Prawns lost weight in both treatments, and mortality was 33% in diatom treatments and 66% in starved treatments. There was a significant difference in the growth of abalone given diced shrimp compared to starvation controls (t-test,  $p < .001$  for abalone length and weight; Fig. 4). Weight of abalone fed only diced shrimp increased 36% over initial weight in 32 days, and length increased at a rate of 73  $\mu\text{m}/\text{day}$ . There was no abalone mortality in either treatment.

## DISCUSSION

Implementation of new aquaculture systems requires experimentation and pilot-scale development prior to commercial appli-

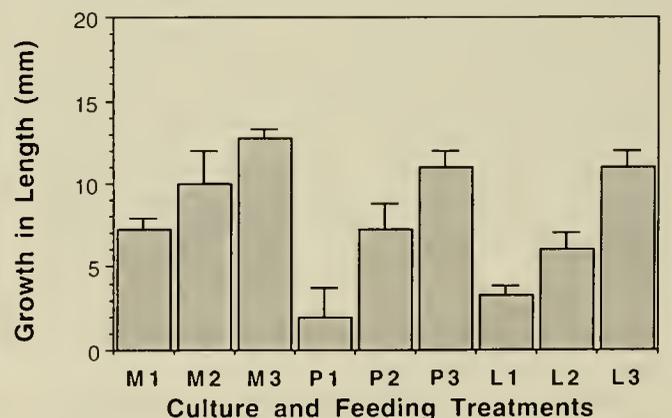


Figure 2a. Growth in length (mm) of juvenile prawns in monoculture (M), polyculture (P), and larger lower density polyculture (L) at 3 feeding rates (1, 2 and 3). Columns indicate mean increase in length ( $\pm$ standard deviation,  $n = 3$ ).

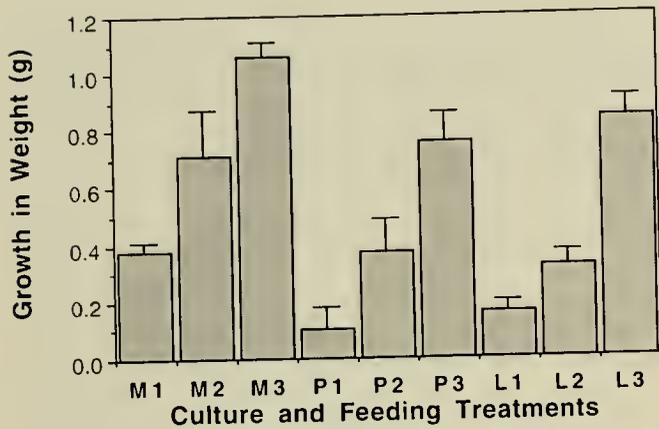


Figure 2b. Growth in weight (g) of prawns in monoculture (M), polyculture (P), and larger lower density polyculture (L) at 3 feeding rates (I, 2 and 3). Columns indicate mean increase in weight ( $\pm$ standard deviation,  $n = 3$ ).

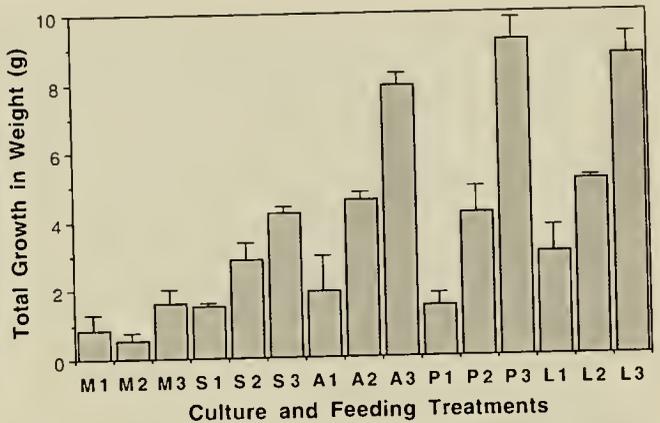


Figure 3. Combined growth in weight (g) of all animals in mussel monoculture (M), prawn monoculture (S), abalone monoculture (A), polyculture (P), and larger lower density polyculture (L) at 3 feeding rates (I, 2 and 3). Columns indicate mean increase in weight ( $\pm$ standard deviation,  $n = 3$ ).

cation (Huguenin and Webber 1981). In this study, we conducted an experimental investigation of biological interactions among 3 species with potential for commercial polyculture. Results demonstrated that there was unsuspected trophic overlap between species, and indicated that while total production might be increased through polyculture, combined culture might not be as effective as a sequential system that maximized resource use but limited species interaction.

In this study, total production of combined shellfish biomass was greatest in the larger polyculture, which had a combined density similar to the density of single species in monoculture (Fig. 3). Total production from the smaller polyculture, in which each species was at its monoculture density, was not significantly different from production of abalone in monoculture. Density, however, did not appear to be a major factor. Density significantly affected only abalone growth (in weight), and the difference in abalone growth was the main contributor to the difference in total production between the 2 densities in polyculture. Since abalone grazed not only

the added diatoms plates but also the organic growth on culture container walls, and since the walls of the larger container had 60% greater surface area, it was likely that the difference in the abalone growth was due primarily to feeding rate as opposed to density. This was also evident from the data showing that the difference in abalone growth rate between the 2 polyculture densities decreased as feeding rate increased, with the 2 becoming essentially equal at the highest feeding rate (Table 1).

The major factor responsible for better abalone and prawn growth in monoculture relative to polyculture was overlap in food utilization by the two species in polyculture. The interaction between culture type and food availability was statistically significant for abalone growth in weight and total production (Tables 1 and 2). Feeding experiments demonstrated that while prawns did not grow on a diatom diet, abalone grew at high rates (73 microns/day) when fed only diced shrimp (Fig. 4). Since each species had access to all 3 foods in monoculture, the growth rates of these 2 species were higher in monocultures than in polycultures, where they had to compete for the diced shrimp.

TABLE 2.

Total production.

Culture System	Food Group:			Mean
	I	II	III	
Mussel Monoculture	0.87 + 0.41	0.53 + 0.25	1.60 + 0.43	1.00
Prawn Monoculture	1.52 + 0.11	2.85 + 0.52	4.23 + 0.15	2.87
Abalone Monoculture	1.93 + 1.05	4.57 + 0.24	7.87 + 0.40	4.79
Polyculture (High Density)	1.48 + 0.35	4.15 + 0.80	9.19 + 0.63	4.94
Polyculture (Low Density)	2.99 + 0.77	5.11 + 0.07	8.76 + 0.63	5.62
Mean	1.76	3.44	6.33	3.84

Values are mean and standard deviation ( $n = 3$ ) for growth in total live weight for all animals in each replicate container. Abalone monocultures had 10 abalone; mussel monocultures had 20 mussels; prawn monocultures had 4 prawns; and all polycultures had 10 abalone, 20 mussels, and 4 prawns. The duration of the test was 100 days.

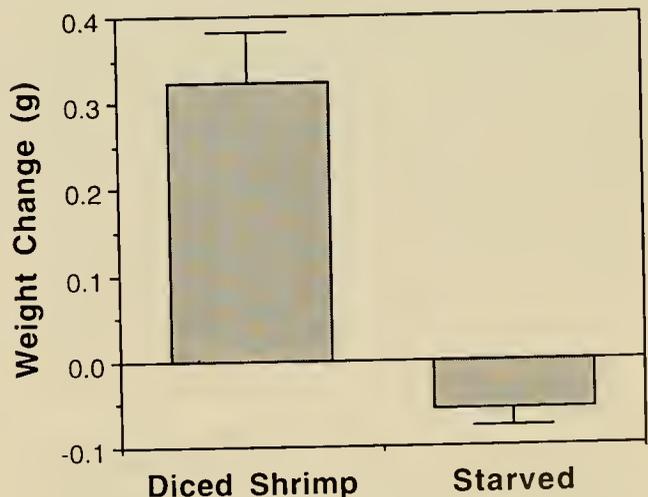


Figure 4. Mean weight increase ( $\pm$ standard deviation,  $n = 3$ ) for abalone fed only diced shrimp and abalone given nothing but filtered seawater for 33 days.

The use of animal food items by abalone has been observed previously. Mottet (1978) mentions incidental consumption of small hydrozoans, copepods, foraminifera, and bryozoans by abalone; VanBlaricom and Stewart (1986) observed black abalone consuming moribund pelagic red crabs; and fish meal and other protein sources are incorporated into artificial diets for abalone (Chen 1985, Uki et al. 1985). The present study, however, offers evidence of exceptional growth by abalone fed only meat.

Clearly, polyculture production was limited by poor growth of bivalves. The combination of unfiltered seawater and 9 hours per week of exposure to phytoplankton was obviously insufficient to sustain acceptable growth rates. Quick seawater turnover times prevented growth of phytoplankton that may have resulted from nutrient enrichment from excreta of other polycultured species. The maximum mussel growth rate, in monocultures at the highest feeding level, was 11 microns/day, compared to reported values of 65 microns/day (Skidmore and Chew 1985), 54 to 67 microns/day (Harger 1970), and 69 microns/day (Tenore et al. 1973, for *M. edulis* in polyculture). Other polyculture systems have demonstrated sufficient bivalve growth when bivalves are held in separate tanks receiving effluent from fish or shrimp ponds (Shpigel et al. 1993). These systems have larger flows and longer retention times than the experimental system described here.

Food may have been limiting for abalone and prawns, as well, since the relationships between growth and feeding rate showed no trend toward stabilizing as feeding rate increased (Figs. 1 and 2). However, abalone growth rates were comparable or higher than values from previous studies. Abalone in monocultures at the highest feeding level grew at a mean rate of 67 microns/day, compared to reported mean annual growth rates for similar sized abalone of 44 microns/day (Owen et al. 1984), 47 microns/day (in polyculture, Tenore 1976), 61 microns/day (Leighton 1974), and 67 microns/day (Ebert and Houk 1984). Feeding rates for prawns were comparable to those used in previous studies. Prawns fed to excess by Kelly et al. (1977) consumed 4.5% of their body weight per day in diced foods, which is within the range of 3 to 10% used in the present study. Prawn growth rates averaged 9 mg/day in this study, lower than reported rates for older and larger *P. platyceros*

individuals: 21 mg/day (Rensel and Prentice 1980), 31 mg/day (Wickens 1972), and 35 mg/day (Butler 1964, from histogram studies of natural populations). Feeding rates in the present study were chosen to cover a range from near starvation to near excess to elicit maximum use of any incidental food items, such as fouling organisms, as part of the polyculture evaluation.

While the success of a polyculture operation is most dependent on the choice of species used (Levandowsky 1977, Gundermann and Popper 1977), the configuration of the culture system is also a key factor. Red abalone, California mussels, and spot prawns have high marker value, similar temperature and salinity tolerances, and a substantial literature on life history and culture. All 3 have been cultured commercially or on a pilot scale, and commercial hatcheries may benefit from integrated culture of the 3 species to increase and stabilize production. The present experimental investigation, however, demonstrated that a mixed culture was impractical because of trophic overlap between prawns and abalone, and perhaps because of water quality considerations in providing suspended food to bivalves. Rather than culturing the 3 species in the same tanks, a sequential system, similar to those previously proposed (Tenore 1976, Mann and Ryther 1977, Shpigel et al. 1993), may be more appropriate. Effluent from prawn cultures drained to sedimentation tanks could provide particulate food to bivalves. Effluent from bivalve tanks could provide nutrients for culture of seaweeds such as *Ulva* (as used by Tenore 1976), which could then be used to supplement feed, an often limiting factor, in abalone culture. Such a system would avoid the negative interactions among species, while exploiting the potential for greater overall production observed in the present study.

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## MOVEMENT, REPRODUCTION AND GROWTH OF OVIGEROUS LOBSTERS (*HOMARUS AMERICANUS*) FROM NEWFOUNDLAND RELEASED OFF GRAND MANAN, BAY OF FUNDY

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**ABSTRACT** During August 1987, 842 Newfoundland ovigerous lobsters, purchased from a commercial holding pound in New Brunswick, were tagged and released off North Head, Grand Manan. This study, prompted by industry speculation on the value of a release program, was to assess their subsequent movement and molt-reproductive cycle in the Bay of Fundy. By March 1991, 317 (37.6%) had been recaptured. Ninety-five percent of these had been recaptured within 10 km of the release site. These lobsters differed from mature Bay of Fundy females in having no apparent long distance deep-shallow seasonal migration, but they had similar reproductive cycle. Recaptured lobsters had hatched the eggs they were carrying when released and had moulted by fall 1988. Two years after release, most recaptures had extruded new eggs, and 3 yrs later most had hatched these eggs and moulted again. Annual molt increments ranged from 11.4% to 8.7% of premolt carapace length (CL) over the 80–93 mm CL range. The uncertainties associated with the release of non-native ovigerous females, purchased from pounds, into the Bay of Fundy to enhance lobster stocks through release and recruitment of larvae are discussed.

**KEY WORDS:** Growth, movement, reproduction, lobster, enhancement, tagging

### INTRODUCTION

Fisheries regulations in Canada do not allow the retention of ovigerous lobsters *Homarus americanus*. This presents a problem to commercial lobster pound owners who may hold lobster for several months prior to marketing (McLeese and Wilder 1964). From June through September, many impounded female lobsters become ovigerous. To counteract this problem, pound owners have the choice to cull and sell females before summer, or purchase special licenses that allow them to either keep ovigerous lobsters in their pounds, or to export them to countries that will accept them on their market. Because of the availability of ovigerous lobsters in Bay of Fundy pounds, the Grand Manan Fishermen's Association requested that a pilot study be conducted to determine the feasibility of purchasing ovigerous lobsters from pounds and returning them to the wild in an attempt to increase larval production.

Enhancement of wild lobster stocks using hatchery-reared larvae or post-settlement stage lobsters has been the focus of several studies in Europe (Howard 1982, 1988, Lorec 1983, Beard et al. 1985, Wickins et al. 1986, Meeren et al. 1990, Meeren and Naess 1991, 1993, Bannister et al. 1994) for *H. gammarus*, and in the New England States (Taylor 1949, Carlson 1954, Van Olst et al. 1980) for *H. americanus*. Some of the more recent studies have shown that hatchery-reared lobsters can survive in the wild to commercial size lobsters (Meeren and Naess 1993, Bannister et al. 1994, Addison and Bannister 1994). Attempts to introduce lobsters in areas where they did not exist previously (Butler (1964), Ghelardi and Shoop (1972) on the west coast of British Columbia, Kittaka et al. (1983) off the coast of Japan), or where environmental conditions are beyond the normal range (Boothroyd and Ennis (1992) off Labrador on the Atlantic Coast) failed to produce viable populations.

Increasing production by returning females that become ovigerous in pounds back into the wild has not previously been examined

as an alternative method for enhancement. In the present study ovigerous lobsters, which had originated from Newfoundland, were obtained from a New Brunswick pound. The rationale behind the use of these animals, rather than those of the native Bay of Fundy stock, was based on their smaller size at maturity (Ennis 1980, 1984a, Campbell and Robinson 1983) and consequent lesser expected cost per animal for any subsequent commercial scale release exercise; were this to be deemed appropriate.

The objective of this study was to assess, under Bay of Fundy environmental conditions, the growth, reproductive cycle and movement of mature Newfoundland females released from pounds. The uncertainties in larval production and the potential problems associated with the release of non-native ovigerous females, purchased from pounds, into the Bay of Fundy to enhance lobster stocks through release and recruitment of larvae are discussed.

### MATERIALS AND METHODS

A source of Newfoundland ovigerous lobster was found in a New Brunswick commercial lobster pound. These lobsters had come originally from the southeastern part of Newfoundland off Placentia Bay and Fortune Bay. Between 11 and 26 August 1987, 842 of these ovigerous lobsters from Newfoundland were tagged and released in Flagg Cove off North Head, Grand Manan (Fig. 1), at depths between 10 and 12 m and bottom temperatures between 9.3 and 10.1°C. Sphyron anchor tags were inserted with a hypodermic needle into the dorsal musculature between the carapace and abdomen of the lobster so that a coded pink vinyl tubing projected dorsally above the abdomen (Scarratt and Elson 1965, Scarratt 1970, Campbell 1986).

Fisherman familiar with past lobster tagging and release studies (Campbell 1983, 1986) cooperated with the tag recovery program. Information on size, sex, reproductive state, location, depth and date of recapture was provided for most lobsters recaptured. In addition, some fisherman from Grand Manan, using vernier calipers (Campbell 1986), recorded size and returned all ovigerous lobsters to the sea with tags still attached.

Movement data was divided into time periods in order to take

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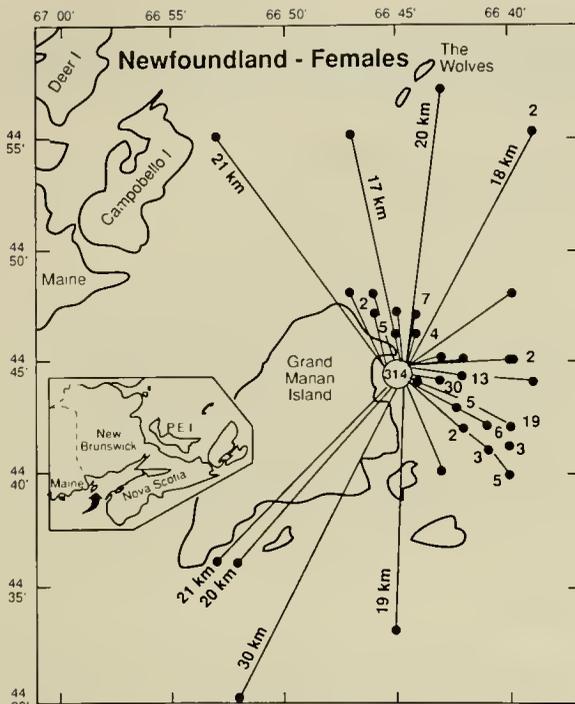


Figure 1. Recapture points and straight-line distances moved by tagged Newfoundland ovigerous lobsters released in Flag Cove off Grand Manan in August 1987. Each point represents one recapture except where otherwise shown. Number in circle is the number of lobsters that were recaptured <2 km from the release location. Inset shows general area.

into consideration sources of bias associated with seasonal lobster fishing patterns. Grand Manan lobster fishing seasons begins the second Tuesday of November and closes the end of June of the following year.

A linear regression (Ricker 1973) was used to fit premolt ( $L_x$ ) and postmolt ( $L_x + 1$ ) carapace length (mm) data:

$$L_x + 1 = a + bL_x$$

where  $a$  and  $b$  are regression coefficients estimated by the least squares methods (Hiatt 1948). Only data collected within 1 yr of release (one molt period) were used in the analyses to prevent any bias caused by double molts. In calculating growth, only lobsters exhibiting growth increases >4 mm CL were used in the analysis because any molts with <4 mm increase were considered measuring errors (Campbell 1983).

Mean distance moved between time periods was compared by a one-way analysis of variance (ANOVA) and Student-Neuman-Keuls tests (Kim and Kohout 1975). Only geometric means are shown, unless otherwise indicated. The direction of lobster movement was analyzed according to Jones (1959), Batschalet (1965) and Saila and Flowers (1968), using a computer program developed by Campbell et al. (1983), and adopting assumptions as in Campbell and Stasko (1985, 1986).

## RESULTS

Eight hundred and forty two Newfoundland ovigerous lobsters were tagged and released (mean size = 84.5 mm CL; range 78–111 mm CL). Three hundred and seventeen individual lobsters

were recaptured: 221 of the recaptures were caught once, 67 twice, 20 three times, 7 four times and 2 five times.

### Movement

More than half of all recaptures was within 2 to 4 months after release, and more than 90% was within 500 days (Table 1). Most recaptures occurred in periods of high fishing effort during fall (November and December), and during spring (May and June) (Campbell and Duggan 1980, Campbell 1992).

Examination of the straight-line distances between release and recapture points indicates that 95% of recaptured lobsters had moved less than 10 km from the release site, and that the mean distance moved (m) per day did not significantly ( $p < 0.05$ ) increase with time (Fig. 1, Table 1). Movements were generally short distances as indicated by a low mean square dispersion coefficient,  $a^2$  ( $0.31 \text{ km}^2 \cdot \text{d}^{-1}$ , Table 2). However, the directional statistics show that there were significant ( $p < 0.01$ ) directed movements by lobsters either south and east or north and east away from Grand Manan (Table 2). The negative east-west vector components (i.e., westerly movement) were undoubtedly influenced by the constraints imposed by the coastline of Grand Manan (Fig. 1).

Most (88%) of Newfoundland lobsters were recaptured at depths <30 m. Only 12% were recaptured at depths between 31 and 60 m and 1 lobster was caught deeper than 60 m.

### Reproductive Cycle

For single recaptures within the first year after release, 98.5% of 208 Newfoundland female lobsters remained ovigerous. None of the 205 females recaptured with size information had moulted since release (Table 3). During the second year, 97.9% of the 142 Newfoundland females recaptured had released their eggs (some possibly lost eggs due to handling) and 99.1% of the 108 females for which there was size information had moulted. During the third year, 11 (84.6%) out of 13 Newfoundland females recaptured were berried and all 7 lobsters with size information had moulted. During the fourth year, none of the 8 females recaptured were

TABLE 1.

Percentage of total recaptures and distance (m) moved per day at large for Newfoundland female lobsters released in Flag Cove, off Grand Manan, during August 1987.

Date (No. of Days at Large)	No. of Tags Returned	Percentage of Total Recaptures	Mean Distance (m)/day (95% Confidence Limit)
Nov. '87-Jan. '88 (82-143)	229	52	22 (20-24)
May '88-June '88 (257-312)	60	14	5 (4-6)
Nov. '88-Dec. '88 (445-489)	124	27	9 (7-11)
May '89-June '89 (623-672)	14	3	11 (3-19)
Nov. '89 (817-826)	9	2	7 (0-15)
Nov. '90 (1186-1193)	7	2	10 (6-13)
Total	443	100	15 (14-17)

TABLE 2.

Directional statistics by month for tagged Newfoundland ovigerous lobsters released in Flagg Cove, Grand Manan, during August 1987.

Month of Release	@	a <sup>2</sup> (km <sup>2</sup> · d <sup>-1</sup> )	V (km · d <sup>-1</sup> )	V' (km · d <sup>-1</sup> )	R	Z	N
Nov. '87	174	0.07	-0.010	0.001	34.4	8.6*	137
Dec. '87	165	0.06	-0.013	0.003	33.1	12.7*	86
Jan. '88	265	0.02	-0.000	-0.002	1.9	0.6	6
May '88	21	0.01	0.004	0.001	33.4	25.4*	44
June '88	344	0.01	0.003	-0.001	11.3	8.0*	16
Nov. '88	140	0.07	-0.005	0.005	51.3	27.1*	97
Dec. '88	106	0.10	-0.001	0.004	7.7	2.2	27
May '89	186	0.21	0.012	-0.001	2.3	0.6	9
June '89	214	0.00	-0.001	-0.001	2.2	1.0	5
Nov. '89	341	0.09	0.005	-0.002	4.3	2.1	9
Nov. '90	151	0.11	-0.004	0.002	1.0	0.2	7
All Recaptures	161	0.31	-0.006	0.002	82.4	15.1*	443

@ = mean vector angle from true north; a<sup>2</sup> = mean square dispersion coefficient; V and V' = non-random movement along north-south and east-west planes, respectively; negative values of V and V' = net southerly and westerly movement, respectively; R and Z = Rayleigh test statistics for randomness of distribution of points about a circle, \* = significance at  $p < 0.01$  indicating a non-uniform distribution (Batschelet 1965); N = sample size.

berried and all 4 lobsters with size information had moulted a second time since release (Table 3).

The Newfoundland lobsters released in ovigerous condition during late summer 1987 had hatched their eggs and moulted once by fall of 1988, extruded new eggs by fall 1989, and released their eggs and moulted a second time by fall 1990 (Table 3).

#### Growth per Molt

During November and December 1988, approximately a year after release, molt increment ranged between 11.4 and 8.7% of premolt CL for the size range at release of 80 to 93 mm CL. Given the narrow size range (80–93 mm CL) no predictive relationships could be generated, rendering comparison to other growth studies in the Bay of Fundy (60–150 mm CL) (Campbell 1983) and off

Newfoundland (52–92 mm CL) (Ennis 1972, Ennis et al. 1986, 1989) impractical.

#### DISCUSSION

This study indicated that Newfoundland lobsters, which had become ovigerous in a Bay of Fundy lobster pound, molted, grew and produced new eggs under Bay of Fundy environmental conditions. The physiological stress on these Newfoundland females caused by transporting them from Newfoundland, holding them in pounds (1 or 2 mo.) and by handling and holding them in crates prior to tagging is unknown. However, despite the stress endured, the percentage of tag returns for Newfoundland females (37.6%) was as successful as could be expected when compared to sphyron tag returns for Bay of Fundy ovigerous lobsters (48.9%, Campbell 1986) and for Bay of Fundy lobsters of both sexes combined (29.3%, Campbell and Stasko 1986). The percentage of returns was also within the 24 to 39% range (1 to 5 years after release) for lobsters of both sexes caught off Newfoundland (Ennis 1972, Ennis et al. 1989). In comparison, during the previous studies tagged lobsters were released within minutes of capture.

The percentage of Newfoundland females (size range of 83–93 mm CL) that had moulted by the second (99%) and third (100%) year of this study was higher than the percentage of native females recaptured during the second (75%) and third (83%) year in Campbell's (1983) study. This molt frequency was also higher than values (range between 90 and 42%) reported for similar size lobsters in several other studies off the coast of Newfoundland (Ennis et al. 1982, 1986, 1989). The main reason for the high molt frequency (99%) obtained in this study probably was that all Newfoundland females were at the same stage of the molt-reproductive cycle when released. Therefore it was highly probable that these females were all going to molt during the following summer after releasing their eggs. In previous studies (Campbell 1983, Ennis et al. 1982, 1986) the molt probabilities were lower because their estimations were based on females taken randomly from wild lobster populations, and which were at various stages of the molt-reproductive cycle.

In the Bay of Fundy, female lobsters can exhibit various molt-

TABLE 3.

Molt-reproductive cycles from recaptured Newfoundland lobsters tagged and released in Flagg Cove, Grand Manan during August 1987.

Time Period at Liberty	No. of Recaptures with Information	% Molted (No. Measured)	% Berried
First year (3–10 mo)	208	0	98.5
Nov. '87–June '88		[205]	
Second year (14–22 mo)	142	99.1	2.1
Oct. '88–June '89		[108]	
Third year (26–37 mo)	13	100	84.6
Nov. '89–June '90		[7]	
Fourth year (38–43 mo)	8	100	0
Nov. '90–Mar. '91		[4]	
Total	371		

Numbers in brackets are numbers of lobsters actually measured.

reproduction schedules. However, in the initial years of maturity, females below 120 mm CL typically exhibit a 2-yr cycle (Waddy and Aiken 1986). Mature Bay of Fundy females molt between the months of July and October, extrude eggs the following summer, and carry them until hatching the next spring, after which they can either molt or extrude new eggs, depending on size (Waddy and Aiken 1986). Based on the tag recovery information, Newfoundland females used in this study appear to have had a reproductive schedule typical of mature Bay of Fundy females of less than 120 mm CL (Campbell 1983, Waddy and Aiken 1986, Robichaud and Campbell 1991).

The benefits of increasing the abundance of ovigerous lobsters in the Bay of Fundy in terms of lobster larval production and subsequent recruitment are unknown. Little is known about the relationship between seasonal movement of adult lobsters (Cooper and Uzman 1971, Krouse 1980, Campbell 1984, 1990, Robichaud and Campbell 1991), planktonic larval movement and settlement patterns (Stasko 1980, Stasko and Campbell 1980, Ennis 1986, Harding and Trites 1988), and oceanographic features. Major concentrations of ovigerous lobsters have to date been found during summer in the upper Bay of Fundy (in Chignecto Bay), and in Flagg Cove and Whale Cove, Grand Manan (Campbell 1990, Lawton and Robichaud, unpubl. data). Concentrations also are known in certain offshore areas, such as the shoal waters of Browns Bank and Georges Bank (Campbell and Pezzack 1986, Harding and Trites 1988). Ovigerous lobsters are assumed to migrate to these areas in the summer to obtain sufficient degree-days to optimize the larvae's chances of survival and assure that they will disperse and settle on a suitable bottom (Campbell 1986, 1990).

Interpretation of movement data obtained from the recovery of tagged lobsters provided by commercial fishermen should be considered with caution due to bias introduced by non-uniform temporal and spatial distribution of fishing effort (Fogarty et al. 1980, Campbell and Stasko 1986, Pezzack 1987). Bay of Fundy lobsters in the vicinity of Grand Manan are known to undertake seasonal deep-shallow migrations (Campbell 1986, 1990). In the present study, although the patterns of fishing effort that intercepted Bay of Fundy lobsters in deep waters over winter in Campbell's (1986, 1990) studies were well placed to catch any Newfoundland lobsters, none were intercepted. No seasonal deep-shallow migration was evident for Newfoundland females, which is consistent with the small-scale movement reported for lobsters off Newfoundland (Ennis 1984b, Ennis et al. 1989).

The Newfoundland ovigerous lobsters were introduced in Flagg Cove during summer on a known summer lobster spawning ground (Campbell 1990). Over a period of 4 yrs, the average displacement from the release location was only 3.8 km (2.0–5.6 km, 95% confidence limit). The pattern of single recaptures, (giving maximum displacements of 20 to 30 km) and the number of single and repeat recaptures (314) less than 2 km, and given the low mean square dispersion coefficient ( $0.31 \text{ km}^2 \cdot \text{d}^{-1}$ ), lead to the conclusion that these Newfoundland lobsters were relatively "local" in their movement. If the majority of Newfoundland females remained close to the area of release, this would increase their chances of mating with local males and releasing their larvae on the same spawning ground as the Bay of Fundy lobsters.

The Bay of Fundy lobster stock is considered to be part of the larger Gulf of Maine stock (Pezzack and Pringle 1986, Pezzack 1987, Harding and Trites 1988, Harding et al. 1993). Introduction of non-local biotype in large numbers, as may be involved in a full enhancement program in the Bay of Fundy, could be of concern for the entire Gulf of Maine lobster fishery. Genetic mixing with native stock could have a negative impact on the adaptability of local biotype to environmental changes. However, genetic mixing of various lobster stocks has been going on in the region since the turn of the century (Harding et al. 1993). Lobster pounds in the Bay of Fundy and in the New England States have been importing lobsters from other areas for many years, and each year unknown numbers of lobsters either escape or are released into the wild. Initiating a large-scale program of release of ovigerous females from pounds may greatly exacerbate the problem of genetic mixing of lobster stocks.

Even if larval survival were successful, it would not guarantee increased recruitment to the fishery. Fogarty and Idoine (1986), using recruitment models, demonstrated that extreme increase in the number of stage IV production did not markedly affect resulting stock size. Therefore, no detectable increase in landings could be attributed to this experiment because of the relatively small number of lobsters released.

Enhancement of wild lobster stocks has had limited success (Conan 1986, Addison and Bannister 1994). With the use of microtagging techniques, recent studies have shown that hatchery-reared animals can survive in the wild and recruit to a fishery (Walker 1986, Wickins et al. 1986, Meeren and Naess 1993, Bannister et al. 1994). However, there remain important biological questions on whether released animals provide sustainable benefits to the fisheries and actually enhance or simply displace natural stocks (Addison and Bannister 1994). At present, the major problem in determining the feasibility of enhancing production of a commercial lobster stock by releasing berried females is that there is no method available to assess larval survival, location of settlement, and the impact on the native populations.

In conclusion, Newfoundland female lobsters released in the Bay of Fundy appear to have molted and grown successfully and followed a similar 2-yr reproductive cycle as described for smaller Bay of Fundy lobsters. However, there is no method available to evaluate larval recruitment success to the natural stocks, and subsequently, the economic feasibility of purchasing ovigerous lobsters to increase recruitment to the lobster fishery in the Bay of Fundy.

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## REDUCTION OF SHELL DISEASE WITH AN EXPERIMENTAL DIET IN A NOVA SCOTIAN LOBSTER POUND

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**ABSTRACT** The effects of an experimental pelleted feed on the incidence of shell disease were preliminarily evaluated in a commercial lobster pound in Nova Scotia, Canada during the 1993–1994 winter pounding cycle. Its performance was compared to a traditional feed which consisted of salted codfish racks. The incidence of shell disease was observed in 7.73% of lobsters fed the pelleted diet, and in 10.39% of lobsters receiving the traditional diet. The results suggest improved nutrition may help impounded lobsters resist shell disease.

**KEY WORDS:** Shell disease, lobster, diet

### INTRODUCTION

A frequent pathologic condition of marine and freshwater decapod crustaceans is shell disease. The disease is marked by the development of lesions of the exoskeleton. Opportunistic bacteria and fungi which degrade chitin are presumed to cause the lesions. Invasion of the exoskeleton by these microbes is thought to be initiated by shell damage. Infection appears to be facilitated by environmental stressors such as poor water quality. Physiological conditions such as molt frequency and nutritional status may also contribute to development of the disease (Sindermann 1989).

Shell disease is an economically significant problem observed in lobsters (*Homarus americanus*, H. Milne Edwards 1837) held in tidal impoundments. It adversely impacts the marketability, as well as viability of infected lobsters. In some tidal pounds, particularly those in southwestern Nova Scotia, 10% or more of the captive stock may be annually affected by the disease.

Because shell disease results in perennial economic losses, development of an effective preventative strategy is warranted. One practical avenue of shell disease prevention in lobster pounds may exist in the use of complete and well-balanced feeds. The development of shell disease has previously been linked to nutritional inadequacies (Fisher et al. 1976), and use of such feeds in other types of crustacean aquaculture has been demonstrated to improve disease resistance (Kanazawa 1983). Currently, most lobsters stored in tidal pounds are fed a diet consisting of herring or salt fish racks. These diets are often variable in quality, and may not provide adequate nutrition for lobsters subjected to the stresses associated with high density confinement (Bayer et al. 1978). A high-quality diet with vitamin and mineral supplements may provide increased resistance to the opportunistic bacterial pathogens thought to cause shell disease.

A preliminary investigation of the relationship between shell disease and nutrition was conducted. The study was designed to compare the incidence of shell disease in impounded lobsters fed

a traditional diet to lobsters which received an experimental pelleted feed.

### MATERIALS AND METHODS

The study was conducted in 2 commercially operating lobster pounds in Shag Harbour, Nova Scotia during the 1993–1994 winter pounding season. The site was chosen due to its previous history of shell disease, and because the regular pound personnel were experienced in detection of the disease.

The construction of the pounds was typical, consisting of a 3-sided concrete dam topped by wooden slat fencing. The 2 pounds were contiguous, with a common section of dam and fence separating them. Because of their proximity and uniform construction, environmental conditions such as water exchange and sediment profiles were assumed to be similar in the pounds. The pounds were different in area, however; one measuring approximately 6350 sq. m, the other, 3500 sq. m.

Lobsters were stocked in each pound during a period from 1 December to 20 December, 1993. All lobsters stocked were obtained from Port Maitland, Nova Scotia. Due to changes in market availability, and the different sizes of the 2 pounds, the stocking times and rates were different for each pound. The larger pound received an average of 5,200 kg on 6 occasions for a total stocking weight of 31,300 kg. The smaller pound received an average of 3,500 kg of 5 separate days for a total stocking weight of 17,500 kg.

In accordance with industry practice, lobsters were routinely inspected by pound employees prior to introduction to the pounds. Injured and weak lobsters were culled from each lot. Workers also observed for signs of shell disease. No lobsters displaying signs of the disease were initially impounded.

A single diet was assigned to each pound. The assignment was determined by the overall supply of the particular diet. Lobsters in the larger pound were fed the control diet which consisted of salted

codfish racks. Lobsters in the smaller pound received the experimental pelleted diet. The formulation for the pelleted diet, and proximate analyses of both the salt fish and the pelleted diet are given in Table 1. Feeding was conducted at a rate of approximately 25 kg feed/t lobsters a day. Feeding rates were decreased as water temperature dropped.

Live lobsters and mortalities were removed from both pounds through regular commercial activity over a period from 7 February to 30 March 1994. Removal was accomplished by means of a diver-assisted suction lift. Rates of removal during this period were highly variable due to weather and market conditions. Approximately 80,000 live lobsters were removed and examined by pound employees for signs of shell disease. Lobsters displaying at least one, 0.5 cm<sup>2</sup> exoskeletal lesion were sorted from unaffected lobsters, separately crated and weighed, and the total weights of each respective group were recorded. Dates of removal and pound were also noted. Dead lobsters were not examined for signs of the disease. Estimation of shell disease prevalence and mortality was based on weights.

Because the study was conducted as part of a commercial operation, lobsters were often removed at rates which may have hindered the ability of pound workers to detect shell disease. To investigate that possibility, 500 individual lobsters were randomly removed by suction lift from each pound on 14 March 1994, and inspected more closely by a single researcher for the presence of any shell disease lesions. Frequency counts of individuals with and without the disease were made to determine if the results of these experimental data were similar to those which were commercially obtained. No mortality data were collected at this time.

Both commercially and experimentally collected data were evaluated using a  $\chi^2$  test for independence (Little and Hills 1978).

## RESULTS AND DISCUSSION

A significant difference in the incidence of shell disease occurred between the 2 treatment groups ( $p < 0.01$ ). This difference was verified by both the commercially and experimentally collected data. Commercial data showed shell disease was present in 7.73% of lobsters which received the experimental pelleted diet, while 10.39% of lobsters that received the traditional diet were affected (Fig. 1). Higher disease prevalences were calculated from experimental data (13% for the pelleted diet and 18% for the salt

TABLE 1.

Comparison of the proximate analysis of traditional salt fish and pelleted diets for feeding to impounded lobsters.

Nutrient (%)	Feed	
	Salt Fish Diet <sup>1</sup>	Pelleted Diet <sup>2</sup>
Crude Protein	26.8	40.8
Crude Fat	0.6	11.0
Total Ash	25.3	16.5
Crude Fiber	0.3	2.5
Moisture	46.7	10.2
Ca:P Ratio	2.1:1.3	2:1

<sup>1</sup> Source: New Jersey Feed Laboratory, Inc., Trenton, NJ.

<sup>2</sup> Source: Zeigler Bros. Inc., Gardners, PA. Ingredients: Fish meal, crab meal, soybean meal, wheat, brewer's yeast, corn gluten meal, fish oil, fish solubles, soy lecithin, vitamin premix with supplemental Vitamin C, trace mineral premix.

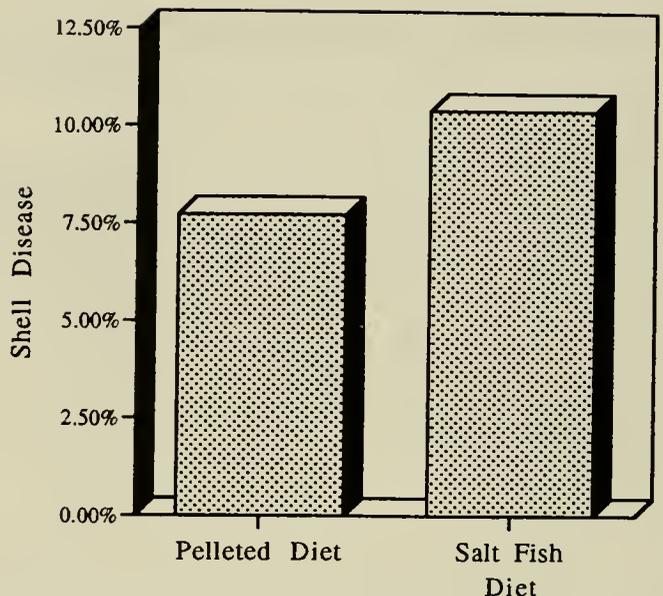


Figure 1. Incidence of shell disease in impounded lobsters with respect to diet. Incidence is expressed as percent of total weight of lobsters recovered from pound.

fish diet), but the trend between treatment groups remained constant.

A significant difference in overall mortality was also noted between groups ( $p < 0.01$ ), with a mortality rate of 4.11% occurring in lobsters fed the pelleted diet, and a rate of 6.47% observed in the control group (Fig. 2).

Since the nature of the study was preliminary, the observed differences in shell disease and mortality must be interpreted with caution. Inherent variability in lobsters and environmental conditions alone may have led to these results. However, there are several possible explanations for the fact that use of the pelleted diet may have resulted in both a decreased incidence of shell disease and mortality. First, due to the lower moisture, lower ash,

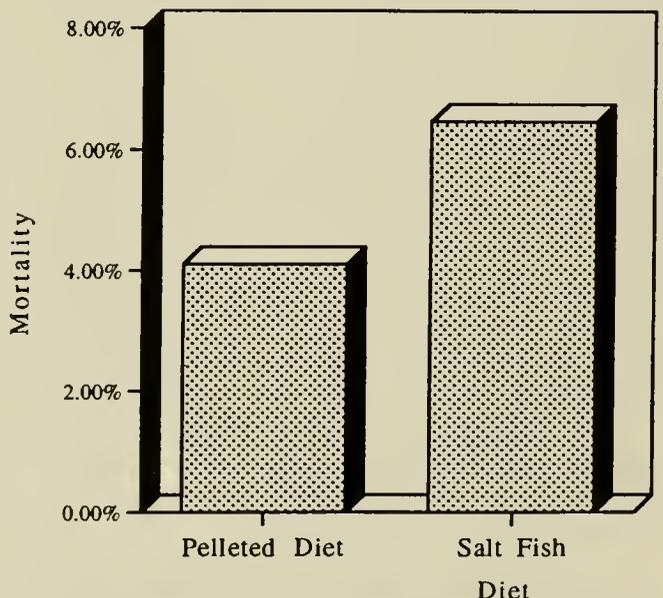


Figure 2. Mortality of impounded lobsters by diet. Values given as percent of initial weight of lobsters stocked in pound.

and higher lipid content, the pelleted diet was more energy-dense than the salt fish. Also, specific nutrients may have contributed to the poorer performance of the salt fish. Although on a dry weight basis the protein contents of both diets were close to one another (44% for the pelleted diet and 40% for the salt fish), the fat content of the salt fish was very low. Lobsters are known to require 1% dietary cholesterol (Castell et al. 1975), and sterol-deficient diets have been associated with decreases in serum protein and calcium, and mean hemocyte counts (Castell and Covey 1976). Finally, the pelleted diet contained additional amounts of vitamins and trace minerals which could also have made it superior to salt fish. Clearly, more experimentation is necessary to verify the results of these feeding trials.

While environmental factors are obviously important, the development of shell disease in impounded lobsters is undoubtedly influenced by the adequacy of the lobsters' processes of cuticular

maintenance, wound repair, and/or internal defense mechanisms. It is reasonable to assume that the experimental diet described here may have provided impounded lobsters with some elements required for these functions that the traditional fish did not, and therefore, conveyed some resistance to the disease. Research to evaluate the role of individual nutrients in these processes, and in the development of shell disease is needed.

#### ACKNOWLEDGMENTS

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## PRESENCE OF THE PHYCOTOXIN OKADAIC ACID IN MUSSELS (*MYTILUS EDULIS*) IN RELATION TO NUTRIENT COMPOSITION IN A SWEDISH COASTAL WATER

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**ABSTRACT** The concentration of the phycotoxin okadaic acid (OA) in mussels (*Mytilus edulis*), collected from the mussel farming district on the Swedish west coast, displayed great geographic variations. The geographical pattern of the variations, however, did not change much during the 5-yr long investigation which started in 1989. Monthly measurements during a 3-yr period of dissolved inorganic nitrogen (DIN), phosphorus (DIP) and dissolved reactive silicate (DSi) were compared with weekly measurements of OA in mussels. Low OA concentrations were found only in a rather isolated fjord system where the highest concentrations of DIN and DIP were also found. The deep water in this fjord was always rich in DSi, and supplied the photic zone with enough DSi to support primary production dominated by diatoms. The DIN/DSi and DIP/DSi ratios in this fjord system were the lowest in the whole mussel farming district. Areas with low DIN/DSi and DIP/DSi ratios during the end of the summer coincided with low OA concentrations in mussels during autumn and winter. High OA concentrations in mussels occurred in areas where DSi was almost totally depleted in July and remained low during the rest of the production season. It is hypothesized that presence of silicate promotes growth of diatoms which are non-toxic here, whereas absence of silicate favors dinoflagellates including DSP-producing *Dinophysis* species.

**KEY WORDS:** Diarrhetic shellfish toxins, okadaic acid, dinoflagellates, mussels, nutrient ratios

### INTRODUCTION

In 1983, 12 yrs after the start of modern mussel (*Mytilus edulis*) cultivation in Sweden, diarrhetic shellfish poisoning (DSP) was observed among people consuming the mussels. For that reason, a surveillance system to detect DSP toxins in mussels was launched in 1986.

The DSP toxin found in Swedish mussels is the phycotoxin okadaic acid (OA) produced by *Dinophysis* spp. (Edebo et al. 1988). A recurring observation was that higher DSP toxin concentrations were found in mussels from the outer archipelago than in mussels from more sheltered waters. Particularly low concentrations (0–14  $\mu\text{g}$  OA  $100\text{ g}^{-1}$  mussel meat) were found in mussels from a fjord system north of the island Orust (Fig. 1) which has a restricted water exchange with sea due to narrow straits and shallow sills (Haamer and Edebo 1990, Edebo et al. 1992).

The seasonal variation in the less sheltered waters often shows a maximum of DSP toxins in the autumn when toxic dinoflagellates are abundant. However, during the spring blooms, normally dominated by diatoms, toxin disappears from the mussels.

During the winter 1989–1990 the harvest of farmed mussels was stopped for a long period due to high OA concentrations. This long closure was one of the reasons for the farmers to look for new areas with less toxins for their farms. The fjord system north of Orust is such an area which was located in 1990 (Fig. 1). In these fjords, the OA concentrations were low (0–14  $\mu\text{g}$   $100\text{ g}^{-1}$  mussel meat) the whole winter in spite of high (100–200  $\mu\text{g}$   $100\text{ g}^{-1}$  mussel meat) concentrations just outside (Haamer and Edebo 1990).

It has earlier been argued that the appearance of OA in mussels in the farming district is caused by inflow of water from the open sea containing toxic plankton (Edebo et al. 1988, Haamer et al. 1990, Edebo et al. 1991). Appreciable concentrations of toxic dinoflagellates in offshore waters have been demonstrated in Skagerrak (Dahl et al. 1985) and off La Rochelle, France (Delmas

et al. 1992). The few observations of increased OA-concentrations in mussels in the sheltered fjords north of Orust occurred in connection with greater inflows of offshore water. However, the fact that the OA-concentrations during these events were also lower in the sheltered fjords than outside, may imply that the phytoplankton composition in the fjords is quite resistant to external influences (Edebo and Haamer, unpubl.).

### Industrial Aspects on the Investigation

The complicated interactions between hydrography, light, nutrients, trace-metals and grazing regulating phytoplankton populations are difficult to measure and interpret. For the aquaculture industry, however, it is a matter of economic survival to be able to predict the probability of appearance of harmful algae in farming waters. For this purpose, we tried to find out if there is a connection between mussel toxins and the composition of plant nutrients in the water. Such a connection should be detected through covariance between the following simply measurable parameters; the concentration of OA in mussels (*M. edulis*) and the concentrations or concentration ratios of DIN, DIP and DSi.

The present study was inspired by results from enclosure experiments (Egge and Aksnes 1992), where the development of phytoplankton communities of different silicate concentrations were studied. Fourteen enclosure experiments during different seasons showed a dominance of diatoms when silicate concentrations exceeded approximately 2  $\mu\text{M}$ . In the experiments, other parameters such as salinity, temperature and solar radiation varied in wide ranges, but these parameters did not show any influence on the results. Egge and Aksnes explained the result of their experiments by the faster growing rate of diatoms compared to other phytoplankton, when silicate concentrations are not limiting (i.e., DSi > 2  $\mu\text{M}$ ). It should be noted that the experiments of Egge and Aksnes were performed not very far from the Swedish mussel farming district and under similar hydrographical conditions.

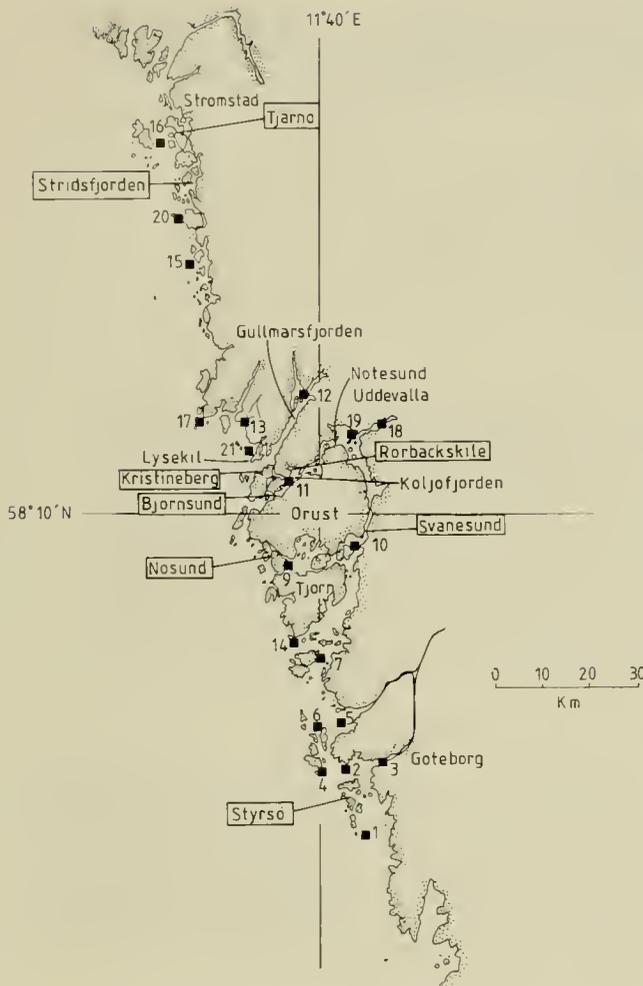


Figure 1. Map of the Swedish mussel farming district. The black squares indicate the positions of the 21 hydrographical and chemical sampling stations (Bohusläns Kustvattenkontroll). Names with a frame are control stations where DSP toxin analyses in mussels are made every week.

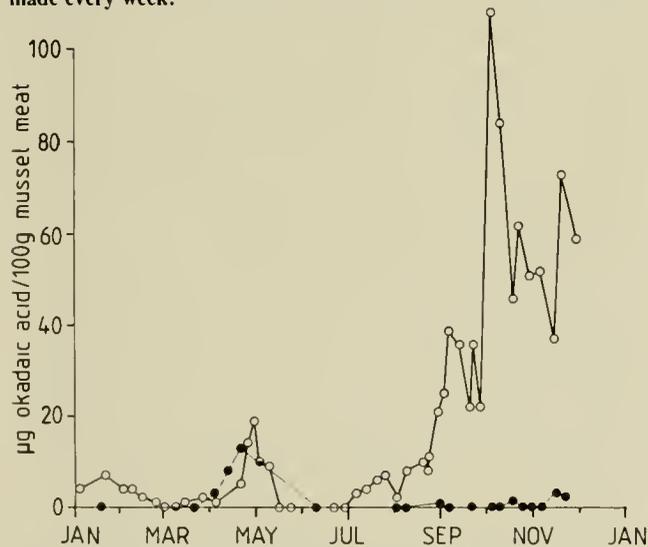


Figure 2. Weekly OA observations at 2 sampling sites in 1993. The black circles represent samples from Rörbäckskile in Koljöfjord which is part of the closed fjord system, and the open circles are for Nösund in the outer archipelago.

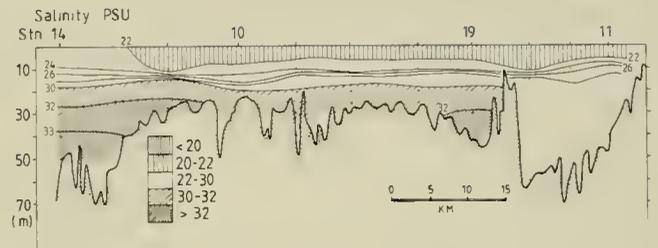


Figure 3. Bottom topography and salinity profile (15 July 1966) in the fjord system around Orust. See Figure 1 for positions of stations.

#### Hydrographic Conditions

According to many authors (Smayda 1980, Sèchet et al. 1990, Delmas et al. 1992) the hydrographic conditions are important for the growth of *Dinophysis* spp. These dinoflagellates are favored by stratified non-turbulent waters, where the relatively slow growing *Dinophysis* spp. can develop in nutrient-poor water and reach cell densities of 15,000 cells/L (Delmas et al. 1992). Along the French Atlantic coast at La Rochelle, the highest concentrations of *Dinophysis* spp. are found where the thermal stratification is strongest, i.e., in offshore waters. Closer to land, the *Dinophysis* spp. concentrations are much lower, which is explained by turbulent mixing, induced by strong tidal currents that do not allow the establishment of thermal stratification.

On the Swedish West Coast, tidal currents are weak and there is also a strong halocline due to the influence of the low saline Baltic water. The strong stratification along the coast is often even stronger within the fjords (Figs. 3 and 4).

#### METHODS

The hydrographical and chemical measurements utilized here, have been done by the Swedish Meteorological and Hydrological Institute, the Swedish Fishery Board, and the Marine Biological Station at Kristineberg and Tjärnö according to standard methods (Axelsson and Rydberg 1993). The sampling area is shown in Figure 1. Samples for nutrient determination were taken at depths 0, 2, 5, 10, 15, 20, 25, 30 and 40 m. The surface concentrations shown here represent the arithmetic mean of all data from 0–10 m except for the stations 2, 3, 5, 7 and 12 (Fig. 1) where the surface concentration is calculated from data obtained at 0 and 2 m. These stations are situated close to river mouths where shallow haloclines are well developed. The calculations in the present paper are mainly based on data from a 3 yr period (1990–1992) with monthly samplings.

Phytoplankton samples were taken more irregularly and only at stations 4, 9, 19 and 16 where the dominant species were counted.

Samples of mussels were collected from at least 6 sites each

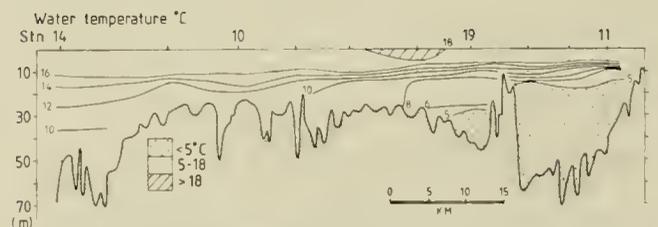


Figure 4. Bottom topography and temperature profile (15 July 1966) in the fjord system around Orust. See Figure 1 for positions of stations.

week (Fig. 1). At Styrösö, Svanesund, Björnsund and Kristineberg, samples were collected from 1 m depth. The other samples were taken from mussel farms where 4 mussels were collected from depth 2, 5 and 8 m. At Styrösö samples have been collected every week since autumn 1989; at Svanesund also since autumn 1989 except for the period May to September; at Nösund since 1988; at Björnsund since September 1990; at Rörbäckskile since August 1992; at Stridsfjorden and Tjärnö since 1988; and at Kristineberg during a period from March 1989 to December 1990; and after that, more irregularly. In the winter 1989–1990, weekly samples were collected from 2 additional sites in the fjord system north of Orust. Also, analyses performed for consumer control of harvested mussels were used. Occasional surveys in certain areas were made for special studies of local differences of OA concentrations in mussels. On these occasions, wild mussels from 0.5–1.5 m depth were collected (Fig. 5). Farming and sampling methods for the Swedish mussel industry have been described by Haamer (1977) and Edebo et al. (1988).

The OA analysis were done after extraction, derivatization with 9-anthryldiazomethane (ADAM), clean up, and HPLC according to Lee et al. (1987) with minor modifications (Edebo et al. 1988).

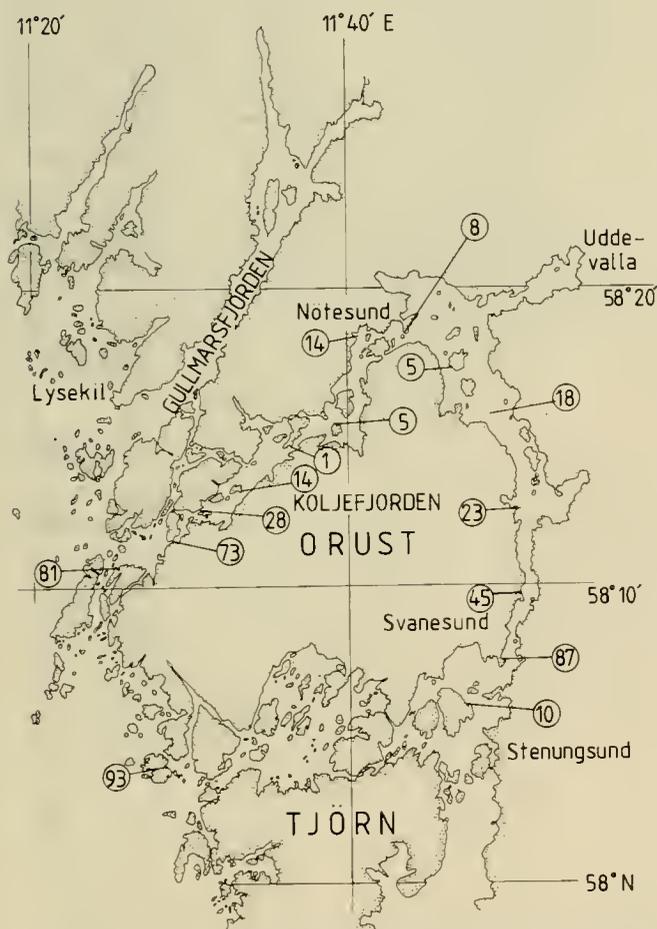


Figure 5. OA concentrations in mussels sampled on 12 October 1993. Numbers within circles indicate OA concentrations ( $\mu\text{g OA}/100\text{ g}$  mussel meat).

## RESULTS

### Topography of and Hydrographical Conditions in the Fjords North of the Island Orust

This fjord system has in the southern end a wide opening to the sea without sills, and water exchange occurs at all depths. North of the island Orust, the fjords are deeper with more narrow openings. The sill depth at Nötesund is 10 m and the vertical cross-sectional area is  $2500\text{ m}^2$ . At the northern opening to the sea at Björnsund, the sill depth is 9 m and the vertical cross-sectional area is only  $450\text{ m}^2$ , which means that water exchange is very limited. The bottom topography along a section through the deepest parts of the fjord system, from station 14 to station 11 (Fig. 1), is shown in Figures 3 and 4. Temperature and salinity profiles from a summer situation (15 July 1966) indicate an increasing vertical stratification from the open sea into the fjord system (Figs. 3, 4). The general circulation in the fjord system is counter-clockwise with a measured net transport of about  $160\text{ m}^3/\text{s}$ . Due to the small extent of deep water exchange, there is nutrient enrichment and oxygen depletion in the basin water at depths below 15 m. This nutrient pool seems to influence the nutrient concentrations in the photic zone during the primary production season. During the summer, the DSi concentrations in the deep water (20–30 m) in Koljefjorden are around  $40\ \mu\text{M}$ , DIP concentrations are around  $1.5\ \mu\text{M}$ , and DIN concentrations are around  $15\ \mu\text{M}$ .

### Seasonal Variation of OA in Mussels

Throughout the years, great seasonal and geographic differences in DSP toxin level in mussels in the farming area have been observed (Table 1) (Edebo et al. 1988, Haamer et al. 1990, Edebo et al. 1991).

There is a general seasonal pattern of the variation of DSP toxins, here represented by okadaic acid (OA) concentrations in mussels along the farming district: if there has been high OA concentrations in the winter, these start to decrease in January. The highest rate of decrease is observed during the spring bloom in late February or early March. The decrease can be as rapid as  $15\ \mu\text{g OA}$  per  $100\text{ g}$  mussel meat per week (observations from Stridsfjorden 1989) (Fig. 1). In the spring, OA normally vanishes presumably due to the fact that mussel food then consists essentially of diatoms. A small increase often occurs in the middle of April but, so far, the OA concentrations at that time have not reached  $20\ \mu\text{g OA}$  per  $100\text{ g}$  mussel meat, the limit for human consumption in Sweden (Table 1). The largest increase of OA in mussels occurs in the beginning of October and the concentrations can increase as much as  $60\ \mu\text{g OA}$  per  $100\text{ g}$  mussel meat in 1 week (observations from Nösund) (Figs. 1, 2).

The pattern described above is a common seasonal pattern for the OA occurrence in the Swedish mussel farming district. However, it is not repeated every year. In the winter of 1992–1993, OA concentrations were low, and the mussel farmers could harvest almost all the season. The following season harvest was terminated in September and the closure lasted until the second week in March 1994 in all areas except one—the fjords north of Orust where mussels were harvested the whole winter (Table 1).

The few algal samples that have been taken in the farming area support data earlier published (Yasumoto et al. 1978, Haamer et al. 1990) that the species causing DSP are *Dinophysis acuminata*, *D. acuta* and *D. norvegica*. These species were found almost every year in small numbers (a few hundred cells/L) in the farming

TABLE 1.  
Periods when mussels could not be harvested due to high OA concentrations.

Year	Tjärnö	Stridsfjord	Björnsund	Nösund	Svanesund
t1988-89	1Sept-20Feb	15Aug-5March		20Aug-20Nov	
1989-90	15Aug-20Feb	10Aug-5April		17Aug-15March	20Aug-1March
t1990-91	1Aug-10Jan	15Aug-1Feb	No	20kt-20Jan	17Oct-20Feb
t1991-92	No	1Dec-20Jan	No	10Dec-17Dec	20Nov-5Feb
1992-93	No	12Sept-20Oct	No	10Sept-25Oct	12Sept-15Oct
1993-94	t2Aug-17Apr	3Sept-15Apr	12Oct-2Nov	27Aug-21Apr	

"No" means no okadaic acid during the whole season. (Sampling stations are indicated in Fig. 1).

area by March. During summer, the cell numbers of *Dinophysis* spp. rose to 10,000 cells/L. This high total count of *Dinophysis* spp. cells was, however, not always paralleled by high DSP toxin concentrations in mussels. During July 1993, *Dinophysis* spp. reach 20,000 cells/L at station 19 and 8,000 cells/L at station 16 without a simultaneous rise of the DSP toxin concentration in mussels taken nearby. The patchiness of the *Dinophysis* populations might explain the discrepancy but other mechanisms might also be involved. This has led to a control program, which is mainly based on analyses of mussels.

#### Geographical Variation of OA in Mussels

The OA concentration in mussels in the outer archipelago increases almost simultaneously along the whole farming area. The Baltic current which is a northward running coastal current has the capacity to spread toxic algae to the sampling sites within 1 week. This means that sampling intervals of 1 week are too long to identify the direction of the "toxin wave." The occurrence of OA in mussels in the fjords can be related to the time for advective water exchange with the coastal water in the different fjord systems.

As the toxins are advected into the fjords north of the island Orust, there seems to be a pronounced decrease in OA concentration in the water mass. This extinction of the toxin wave is reflected in the decrease of OA concentration in mussels (Fig. 5). Weekly monitoring at 9 stations in the fall and winter 1989-1990 (Edebo et al. 1991) showed the same pattern as described above.

#### Variations in Silica Concentrations

Annual cycles of DSi in the surface water at the hydrographical stations in the farming district are shown in Figure 6. Almost all DSi in the outer archipelago has been consumed in July-August while DSi is still left in the fjords north of Orust at stations 18, 19 and 11 (Figs. 1 and 6). The spatial differences of the ratios between DIN, DIP and DSi indicate great differences of growth conditions for phytoplankton in the fjords compared to the outer archipelago (Figs. 7 and 8). Before the spring bloom, DIN:DSi:DIP ratios are approximately 16:15:1 (by atoms) at most of the stations, but the ratios diverge greatly during the summer. At stations 17, 21 and 13 in the outer archipelago, the DIN:DSi ratios are 5.2, 2.6 and 2.8 respectively in summer (Fig. 8). DSi is almost totally depleted in summer while there is still DIN and DIP left to support growth of non-diatoms. In the fjords north of Orust at stations 18, 19 and 11 the DIN:DSi ratios are 0.10, 0.08 and 0.12 respectively and there is more than 3  $\mu\text{M}$  DSi/L in the photic zone during July and August (Figs. 6 and 8). This should be enough to support a production dominated by diatoms (Egge and Aksnes 1992) for the rest of the phytoplankton growing season.

#### Phytoplankton Composition

According to the hydrographic situation in the fjords in autumn, the conditions for *Dinophysis* growth in the fjords are good. In August-September 1993 both the concentration of diatoms and

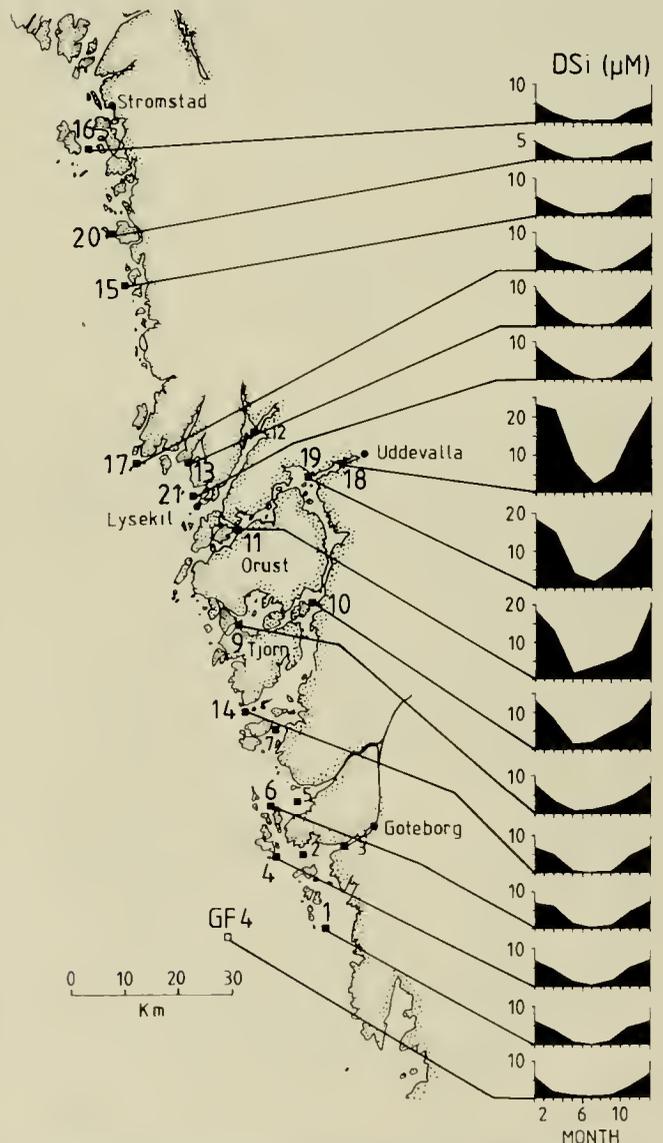


Figure 6. Dissolved silicate (DSi) concentrations in surface water (0-10 m) at the hydrographic sampling stations (Fig. 1). The sampling was carried out in the beginning of each month. The concentrations represent arithmetic means for a 3-yr period from 1990 to 1992 (Axelsson and Rydberg 1993).

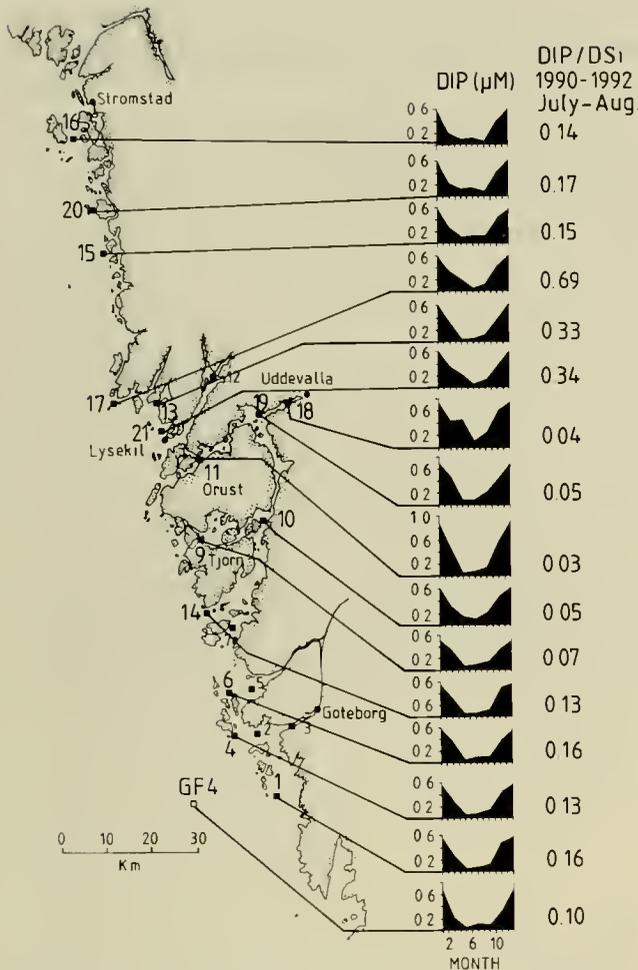


Figure 7. Dissolved inorganic phosphate (DIP) concentrations in surface water (0–10 m) at the hydrographic stations. The samples are taken in the beginning of each month. The concentrations represent arithmetic means for a 3-yr period from 1990 to 1992 (Axelsson and Rydberg 1993). DIP:DSi ratios for July and August during the same period are noted at each station.

of dinoflagellates were higher in the inner fjord system north of Orust (station 19) than in the outer archipelago (station 16) (Table 2). However, the numerical ratio diatoms to dinoflagellates at station 16 (outer archipelago) during August and September 1993 was at least one order of magnitude less than at station 19 (fjord). These data suggest that it is rather the ratio of diatoms to dinoflagellates than the absolute amount of *Dinophysis* spp. that affects the OA concentration in mussels. Alternatively, the variations of OA concentrations in mussels might be caused by variations in *Dinophysis* toxicity (Lee et al. 1989).

**Comparison of OA in Mussels and DSi Concentrations**

September and October are the months when OA concentrations have usually started to rise to high levels in mussels along the farming district. At that time, the DSi has also been depleted at several sites. The average DSi concentrations in the surface layer at the different sampling stations and the OA concentrations in mussels for these months, during the 3-yr period 1990–92 is sig-

nificantly negatively correlated. Thus, low OA concentrations are found together with high DSi concentrations (Figs. 9 and 10).

The prerequisite to obtaining a dominance of diatoms in a phytoplankton population is that the DSi concentration is at least 2 μM (Egge and Aksnes 1992). This criterion is satisfied during fall only at stations 3, 2, 10, 18, 19 and 11 (Figs. 1, 6 and 9). Among these stations, 5, 3 and 2 are influenced by the large Göta River which explains the high DSi concentrations. The OA concentrations in mid October 1993 gives a picture of an average fall situation (Fig. 9). In the fjord area at stations 18, 19 and 11, where DSi concentrations are around 5 μM, the OA concentrations have been low at all sampling occasions. In the southern end of the Orust fjord system (station 10), the OA concentrations in mussels are high although DSi concentrations are also high in the water mass. This exceptional condition in our investigation might be due to shortage of time for the development of a ‘fjord’ phytoplankton population as this is the area where the off-shore water enter the fjords.

**DISCUSSION**

**Lowest DSP Toxin Concentrations in the Most Eutrophicated Waters**

The fjords around Orust and Tjörn are the most nutrient-enriched waters in the whole mussel farming area of Sweden

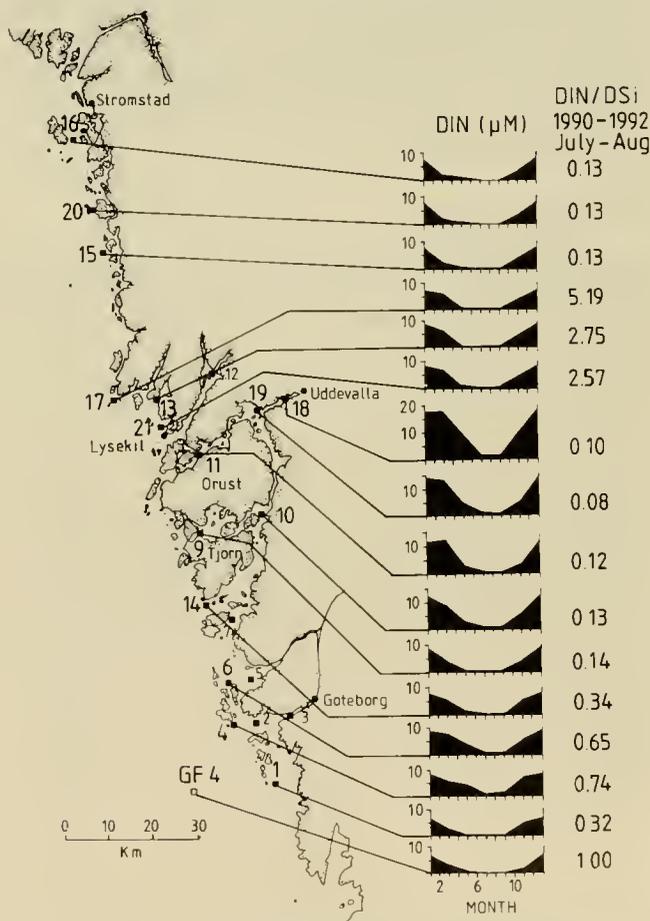


Figure 8. Dissolved inorganic nitrogen (DIN) concentrations in surface water (0–10 m) at the hydrographic stations. The samples are taken in the beginning of each month. The concentrations represent arithmetic means for the 3-yr period from 1990 to 1992 (Axelsson and Rydberg 1993). DIN:DSi ratios for the months July and August for the same period are noted at each station.

TABLE 2.

Diatom and dinoflagellate numbers and ratios in the outer archipelago (station 16) and the inner fjord system (Station 19) on two occasions; DSi concentrations are also given.

Location	Month	Dinoflagellates Cells/L	Diatoms Cells/L	Ratio:Diatoms: Dinoflagellate	$\mu\text{mol DSi/L}$
Station 19	9308	$8.7 * 10^4$	$6.94 * 10^5$	8	2.7
Station 16	9308	$1.6 * 10^4$	$1.1 * 10^3$	0.07	1.1
Station 19	9309	$1.34 * 10^5$	$6.69 * 10^6$	50	7.6
Station 16	9309	$3.13 * 10^4$	$1.79 * 10^5$	5.7	1.2

(Figs. 6, 7 and 8). Therefore it was surprising to find the lowest OA concentrations in mussels in this area (Fig. 9). At first sight these findings seemed to contradict the conclusion that nutrient enrichment favors algal blooms, including toxic algal blooms, as suggested by several authors (Shumway 1989, Daniel et al. 1993). The very special conditions, with high DSi concentrations in the basin water that supply the photic zone with enough DSi to sustain a dominance of diatoms, might be an explanation. Another possibility, which has not been investigated here, is that the nutrient composition in these fjords induces *Dinophysis* to produce less toxin per cell.

Since toxin concentrations have remained low in this area throughout the observation period, i.e., for 5 years, mussel farms have now been established there. Also, wild mussels have been harvested in these fjords when harvest is forbidden in the outer archipelago. Furthermore, during the winter of 1993 and 1994, this area was successfully used for detoxification of OA-contaminated mussels.

#### Inter-annual OA Variations

The inter-annual variations of OA concentrations in mussels along the farming district are large. In some years the whole area, except the mentioned fjords, are closed for harvest during 6 months whereas in other years there are just very short interruptions of the harvest (Table 1). The reasons for these variations are unknown. One hypothesis is that the variations depend on large scale hydrographic conditions along the coast. As this is a confluence area for water from the Baltic, Kattegat, Skagerrak and the North Sea, the weather over Northern Europe is of greatest importance to the movements of those different water masses. So far, however, no serious attempts have been made to correlate weather events to variations in OA concentrations in mussels.

Also, a comparison between DSi concentration in the farming

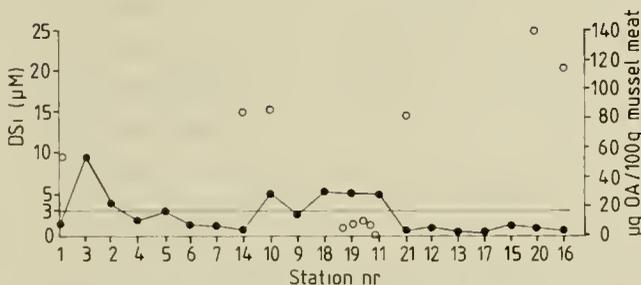


Figure 9. The spatial variations of average surface (0–10 m, 0–2 m) DSi concentrations at stations 1–21 (Fig. 1) during September to October 1990–92 (black circles). The open circles show location and OA concentration in mussels sampled in mid-October 1993.

area close to the open sea, during fall 1990, a season with high OA concentrations, and fall 1992, a season with low OA concentrations, gave no significant differences in the DSi concentrations which were low in the fall during both years in the outer archipelago. These variations in OA concentrations might be related to variations in hydrographic conditions that also regulate the growth of *Dinophysis* spp.

In the outer archipelago, the DSi concentrations reach  $2 \mu\text{M}$  in the first part of October. The fact that OA concentrations in mussels in some years have also increased there at that time (Haamer et al. 1990) contradicts our theory. An explanation to this discrep-

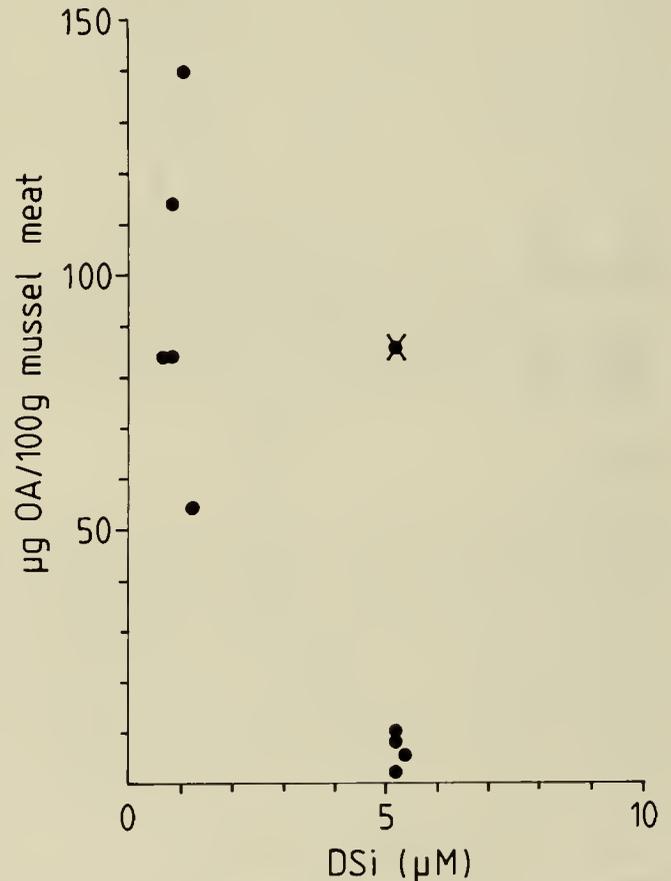


Figure 10. Average DSi concentrations in September to October 1990–92 in the investigated area compared with OA concentrations in mussel samples (black circles) collected close to the hydrographic stations in mid-October 1993. The sample with the cross is from the mouth of the Orust fjord system which is a mixing area for offshore water.

ancy can be that the phytoplankton production season is over then, due to lack of light, and because *Dinophysis* spp. dominate the phytoplankton population due to mixotrophy.

#### Changes of Nutrient Ratios in Kattegat

During the last 30 yrs the ratio between DIN and DSi in the winter surface water of northern Kattegat has changed from 0.5 to 1.1 (Fig. 11). In the same water the DIN concentration increased by 100% between 1971 and 1982 while the DSi concentration decreased (Andersson and Rydberg 1987). DSi is now depleted earlier in the growth season than before in the photic zone in offshore waters along the Swedish West Coast, and DSi now seems to become the limiting nutrient for diatom growth in offshore waters already in May.

#### Historic Occurrence of Diarrhetic Shellfish Poisoning (DSP)

Experimental mussel farming started in Sweden in 1971. After 10 yrs of development work, commercial farming was started. During the whole development period, 1971–1980, mussels were harvested all-year-round. No cases of diarrhetic shellfish poisoning (DSP) were reported until 1983, when the first DSP cases were reported to the health authorities (Edebo et al. 1988, Haamer et al. 1990). Short periods of DSP might have occurred before 1983 without being noticed, but long periods of toxicity, which commonly occur now, should have been noticed. Historically there are no data indicating that there have been any long periods of toxic mussels in Sweden. Incidents of DSP might, however, have been misdiagnosed as bacterial diarrhoea since DSP was described adequately already in 1901 (Thesen).

In other marine ecosystems there are also indications of an increase in DSP events at the same time, or somewhat earlier (Yasumoto 1978). If changing nutrient ratios among the major nutrients is a cause of the increase of DSP cases, a critical change of the macro nutrient composition in Swedish waters should have happened around 1980. In 1980 the ratios DIN:DSi:DIP were 12:15:1 (by atoms) in the Kattegat (December–February) winter surface water. In 1993 the ratios DIN:DSi:DIP were 12:10:1 (Fig. 11).

#### What About the Future for Marine Aquaculture?

The most serious problem, for the mussel farming, other types of aquaculture, and probably also for the wild fishery, is that the DIN and DIP concentrations are still increasing in the open sea along the Swedish west coast while DSi concentrations are decreasing (Fig. 11). What kind of dinoflagellates are going to dominate in the future? The consequence of these unpredictable changes in phytoplankton communities in the open sea, is that mussel and oyster farmers in many areas have to bring their products to depurate for weeks in controlled natural or artificial water basins before delivery.

The discovery of favorable conditions for mussel farming in the semi-closed fjord system north of Orust with its silicate rich basin

#### NORTHERN KATTEGAT SURFACE WATER DECEMBER–FEBRUARY

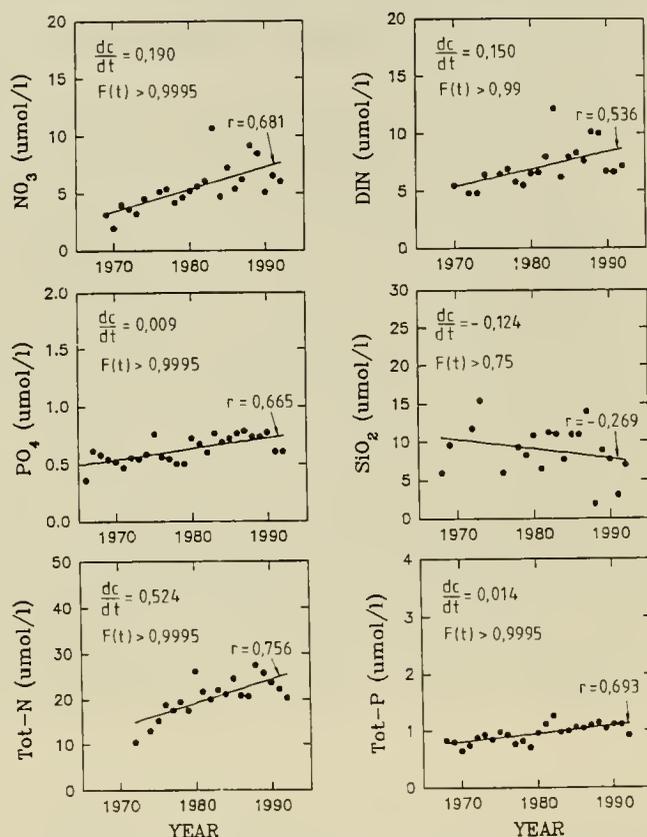


Figure 11. Trends in the nutrient concentrations in North Kattegat (lat. 57°49'–56°50'). The rate of change  $dc/dt$  ( $\mu\text{mol}/\text{yr}$ ) the correlation coefficient,  $r$  and significance level  $F(t)$ , are indicated (Andersson 1993).

water, will probably help the Swedish mussel farmers to survive. But for how long?

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## THE DETECTION AND DISTRIBUTION OF THE MARINE NEUROTOXIN DOMOIC ACID ON THE PACIFIC COAST OF THE UNITED STATES 1991-1993

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**ABSTRACT** Analysis of 2873 samples from a wide variety of domestic as well as import seafoods for domoic acid revealed the presence of the toxin in specimens of anchovies, razor clams, crab, and spiny lobster harvested from the coastal waters of California, Oregon and Washington from 1991 to 1993. Of the 392 razor clam samples investigated, 42% were found to contain the toxin in excess of 20 ppm, a level considered to be unsafe for human consumption by the Food and Drug Administration (FDA). In sharp contrast to the razor clam results, neither mussels nor oysters gathered from the same sampling sites showed any significant sign of domoic acid contamination. Of the total of 397 cooked crab samples harvested from the coastal waters of Washington and Oregon, greater than 65% were found to contain some level of domoic acid and greater than 5% were found to contain levels of the toxin in excess of 20 ppm. These studies also revealed that, in general, crab viscera contained domoic acid at levels 5 to 10 times higher than those found in the meat. While all samples of crab and lobster were analyzed as cooked specimens, all other species investigated were analyzed in the raw state. Of all the species investigated, anchovies were found to be the most highly contaminated.

**KEY WORDS:** Domoic acid, amnesic shellfish poisoning (ASP), neurotoxin, razor clams, anchovies, crab

### INTRODUCTION

Extracts of the seaweed, *Chondria armata*, have been used in Japanese folk medicine as a remedy for infestations of intestinal worms. The active ingredient appears to be domoic acid which was first identified in seaweed extracts (Takemoto and Daigo 1958) some 30 yrs ago. Despite the fact that domoic acid in seaweed extracts has proven to be effective in killing the worms in children it is now recognized as a toxin with major health implications. This recognition stems from the first documented outbreak of domoic acid toxicity in humans (Wright et al. 1989, Teitelbaum et al. 1990) which occurred in December 1987, in Canada on Prince Edward Island (P.E.I.). In that incident, 3 elderly people died and 153 persons became acutely ill from eating domoic acid contaminated cultured blue mussels (*Mytilus edulis*), harvested from the Cardigan River area in eastern P.E.I. The symptoms of mild domoic acid poisoning are reported as abdominal cramps, diarrhea and nausea. More acute symptoms usually appear within 48 hours and include headache, dizziness, facial grimaces, confusion, disorientation, excessive bronchial secretions, breathing problems, loss of short term memory, seizures, coma and ultimately death.

The P.E.I. episode prompted the FDA to issue a directive entitled, "Domestic Fish and Fishery Products Inspection Assignment (FY 91)" to the field on 21 February 1991, to initiate a surveillance program for the toxin in seafood samples being analyzed for paralytic shellfish poisoning. A subsequent incident in early September 1991 in Monterey Bay, CA involving dying Pelicans and Cormorants reinforced the FDA's concern over the possible emergence of a serious public health hazard. With the de-

velopment of suitable regulatory methodology, based on Quilliam's analytical approach (Canadian NRC Technical Report #64, NRCC 33001), an extensive monitoring program began in FDA's Seattle District. To determine the cause of the apparent poisoning, samples of stomachs and stomach contents from the dead seabirds were analyzed by the California Department of Health Services Sanitation and Radiation Laboratory for PSP by the standard mouse bioassay. The dying mice exhibited symptoms which were atypical for PSP, but characteristic for those described (Iverson et al. 1989, Tryphonas et al. 1990, Tasker et al. 1991) for domoic acid (del Rosario et al. 1991, Fritz et al. 1992, Work et al. 1993). At almost the same time that domoic acid was being characterized as the toxin responsible for poisoning the seabirds in California, unusual domoic acid-like symptoms (scratching syndrome) in the mouse bioassay were also detected by analysts at the Washington State Department of Health in monitoring samples of razor clams (*Siliqua patula*) for PSP. Thus, on Veterans Day weekend in November 1991, the Seattle FDA laboratory was asked by the Washington State Health Dept. to analyze several samples of razor clams for domoic acid. The analysis of 3 samples of razor clams revealed levels of 33, 26 and 11 ppm respectively (average 23 ppm) domoic acid. These initial findings were confirmed by M. Quilliam at National Research Center (NRC) in Canada (Private Communication) and by comparison of retention time data and spectra matching in the Seattle lab. The confirmed findings prompted a wider sampling plan and an immediate alert of public officials in the states of Washington, Oregon, Alaska, and California. In the 2 yrs of studies encompassed by this report, 2873 analyses for domoic acid were performed by FDA laboratories on a wide variety of domestic as well as imported seafood samples.

The results confirmed that domoic acid contamination occurred on the west coast during the course of these studies as indicated by the high levels of toxin found in razor clams, crab (*Cancer magister*, *Callinectes sapidus*, *Cancer pagurus*, *Menippe adina*), and anchovies (*Anchoa mitchilli*, *Engraulis mordax*). No apparent problems were detected with any of the imported items.

## MATERIALS AND METHODS

Solvents, LC grade, were purchased from E M Science, Gibbstown, NJ, or Fisher Scientific, Fairlawn, NJ. Analytical grade reagents were purchased from either J.T. Baker Chemical Co., Phillipsburg, NJ or Sigma Chemical Co, St. Louis, MO. Water was deionized in-house. Small quantities of purified standard domoic acid were obtained from Sherwood Hall, U.S. Food and Drug Administration, Center for Seafood Toxin Research, and small quantities of purified domoic acid standard, DACS-1, and domoic acid reference Material, MUS-1, were purchased from National Research Council of Canada. Shellfish [mussels (*M. edulis*), clams (*S. patula*, *Saxidomus giganteus*, *Prothaca staminea*, *Mya arenaria*), oysters (*Ostrea lurida*), crab, sea urchins (*Strongylocentrotus purpuratus*) and finfish (anchovies, sole (*Microstomus pacificus*, *Pleuronectes vetulus*), salmon (*Oncorhynchus* family), perch (*Sebastes* family), etc., miscellaneous marine animals (barnacles (*Balanus spp.*, *Lepas psittacus*), leather kayton (*Katharina tunicata*), etc.] were obtained as samples from surveillance of processor inspections or purchased at retail outlets.

### Sample Preparation

Shellfish were shucked and the tissue composited with a food grinder. Finfish were either analyzed as whole samples or were gutted and the viscera analyzed separately from the flesh. Crabs were shucked and the viscera kept separate from the meat tissue. In some cases the hepatopancreas (mustard) was analyzed separately.

### Sample Extraction

Sample preparation in general followed the method of Quilliam et al. (1991), except that a 1:10 dilution of the extract was made to prevent overloading the solid phase extraction columns used for the final cleanup. Briefly, a 5.0 gm sample of composite was placed in a 50 mL centrifuge tube, 10 mL methanol added, and enough distilled water to attain a final total volume of 20 mL. The sample was homogenized using a Polytron tissue homogenizer for 2 min at 10,000 rpm, then centrifuged for 10 min at 2000 rpm. Ten mL of supernatant were transferred to a 100 mL glass stoppered mixing cylinder and methanol:water, 1:1, added to achieve a final volume of 100 mL. After thorough mixing the solution was loaded onto a conditioned SPE-SAX cartridge.

### SPE Clean-up

Solid phase extraction (SPE) was performed on a Supelco SPE-SAX or Bakerbond quaternary amine cartridge as described by Quilliam et al. (1991). Of the resulting column eluate, 20  $\mu$ l, was injected into the HPLC.

### HPLC Analysis

The LC system consisted of a Shimadzu LC-600 Pump, Shimadzu SIL-9A Autoinjector, Shimadzu SPD-6A Spectrophotometric Detector, and a Shimadzu CR 501 Chromatopac Integrator recorder. The guard column was a Phenomenex, and the column was a Phenomenex Spherisorb 5 ODS (1) 15 cm  $\times$  4.6 mm. The mobile phase used was a v/v/v mixture of water, 83.4: acetonitrile, 16.5:trifluoroacetic acid, 0.1. Conditions: ambient temperature, 20  $\mu$ l injected, UV Detector, 242 nm, flow rate of 1.0 mL/min. Confirmatory HPLC (Burke et al. 1991) was run on a Hewlett-Packard HP 1090 with diode array detector and Chem Station with a 25 cm  $\times$  4.6 mm Phenomenex Spherisorb 10 ODS (1) with a Preplex C-18 guard column. The authors later found that increased sensitivity could be obtained by using a Meta Chem 5  $\mu$  150 mm  $\times$  2.0 mm Spherisorb ODS (2) millibore HPLC column with SAFE GUARD Meta Chem 5  $\mu$  C-18 guard column. The mobile phase was A = 0.75% phosphoric acid, B = tetrahydrofuran, C = acetonitrile with a flow rate of 0.2 mL/min. Domoic acid was eluted using a gradient consisting of Time 0 min = 100% A, Time 5 min 86% A, 7% B, 7% C, held to Time 12 min then 100% A to Time 16 min. Injection volumes were 25  $\mu$ l and a column temperature of 38°C was maintained.

## RESULTS

The domoic acid surveillance program was initiated in 21 February 1991. The samples collected along the Washington and Oregon coast were obtained from the same sites that were normally used for sample collection to monitor for PSP toxin under the National Shellfish Sanitation Program. The first samples received in August 1991 were oysters, but no domoic acid was found. The program was expanded in November of that year to include samples of razor clams and other species, and is ongoing. Of the 392 samples investigated from November 1991 through September 1993, 164 were found to contain domoic acid at levels higher than 20 ppm, 219 at levels between trace and 20 ppm and 9 were found to have no measurable amount of domoic acid (Table 1). Over the same time frame and even during the same collection trips, samples of mussels and oysters were also harvested and tested. Unlike razor clams, both mussels and oysters showed little evidence of contamination by domoic acid. For example, of the 143 samples of

TABLE 1.

Analyses of razor clams collected from the Washington and Oregon coastlines between October 1991 and December 1992.

County	# Analyses	# Positive	# >20 ppm	Range
Clallam	2	2	0	4-24
Jefferson	5	5	3	4-46
Gray's Harbor	87	86	24	ND-100
Pacific	95	89	61	ND-93
Clatsop	148	147	62	ND-124
Tillamook	6	0	0	ND
Lincoln	27	26	10	ND-39
Lane	1	1	0	13
Douglas	15	15	3	5-31
Coos	11	11	1	5-21
Curry	1	1	0	11

ND = Nonc Detected.

TABLE 2.

Analyses of mussels collected from the Washington and Oregon coastlines between October 1991 and December 1992.

County	# Analyses	# Positive	# >20 ppm	Range
Clallam	17	4	0	ND-15
Jefferson	1	0	0	ND
Gray's Harbor	4	0	0	ND
Pacific	10	0	0	ND
Clatsop	7	1		ND-Trace
Tillamook	15	1		ND-Trace
Lincoln	24	1		ND-Trace
Lane	7	0		ND
Douglas	18	0		ND
Coos	14	0		ND
Curry	26	4		ND-1

ND = None Detected.

mussels investigated, only 11 were found to contain any toxin, and only one had a level (15 ppm) higher than 1 ppm (Table 2). Similarly, of the 109 samples of oysters from Washington, only 7 were found to contain trace levels (<1 to 2.2 ppm). No domoic acid was detected in any of the 43 oyster samples taken from Oregon coastal waters, or from the 2 samples taken from Hawaii. However, these results must be interpreted with caution. In the California monitoring program, high levels in excess of 20 ppm were found in mussels from Monterey Bay in November 1991. This time period corresponded with the highest densities of *Pseudonitzschia australis* observed in Monterey Bay. Together these results suggest that while mussels may depurate domoic acid rapidly (Novaczek et al. 1992) they can accumulate high concentrations of the toxin, as evidenced by the P.E.I. incident, and thus pose a serious public health concern.

During the same collection period (November 1991 to September 1993) numerous samples of crab and crab viscera were also obtained off the Washington and Oregon coasts. Because of the nature of crab harvesting, it is more difficult to obtain specific harvest area data. Nevertheless, a meaningful picture emerges from the analysis. Of a total of 281 Dungeness crab viscera from coastal waters off Washington State, 67% were found to contain domoic acid, 4.6% of which were found to contain domoic acid at levels higher than 20 ppm (Table 3). No domoic acid was detected

in 47% of the crab meat samples while the remaining 53% had levels ranging from trace to 6 ppm. Similarly for Dungeness crab harvested from Oregon coastal waters, of 116 crab viscera samples analyzed, 84.5% contained domoic acid approximately 2% of which exceeded 20 ppm (Table 3). No domoic acid was detected in 43% of the crab meat samples while the remaining 57% had levels ranging from trace to 2 ppm. Small numbers of samples of various crab species (Blue *C. sapidus*, Dungeness *C. magister*, Rock *C. pagurus*, and Stone *M. adina*) and spiny lobster (*Palinurus elephas*) from California were also analyzed for the toxin. Of the 4 blue crab samples one was found to contain the toxin (1.1 ppm), of the 8 Dungeness crab samples all were found to contain domoic acid, of the 86 Rock crab samples 54 were positive; 9 of which exceeded 20 ppm, and 3 of which exceeded the FDA action level of 30 ppm.\* Of the 8 Stone crab samples analyzed, 5 were found to be positive 2 of which exceeded the 30 ppm FDA action level and finally of the 20 samples of spiny lobster taken from the vicinity of Catalina Island, 11 were found to contain the toxin at levels ranging from trace to one sample, collected 11-1-92, which was found to contain the toxin at a level of 24 ppm. Lobsters have not previously been reported to contain domoic acid.

Raw anchovies were also surveyed for domoic acid as a consequence of finding the toxin in the stomach contents of pelicans and cormorants that were dying of unknown causes in California's Monterey Bay (Work 1991). Of the 77 samples investigated from California, 58 (75%) proved to contain domoic acid 45 (77%) of which were in excess of the 20 ppm FDA action level (Table 4). As with the crab samples, the anchovy analyses were divided between viscera and meat and similar to the crab, viscera were invariably found to contain higher levels of the toxin (Table 5).

A number of different import species (Table 6) were surveyed for the toxin and no sample was found to be contaminated. Salt interferes with the usual sample cleanup procedure and work will need to be done to find a reliable method for handling salted product since many imported as well as some domestic samples are processed with salt.

\*On 27 January 1993 the FDA issued a directive from Division of Federal-State Relations adjusting the action level of domoic acid in Dungeness crab viscera from 20 to 30 ppm but retained the 20 ppm action level for all other species.

TABLE 3.

Summary of analyses of Dungeness crab collected from the Washington and Oregon coastlines between October 1991 and December 1992.

State	Product	# Analyses	# ND	# Positive	% Positive	Hi ppm	Total >20 ppm	% >20 ppm
Washington	meat	64	30	34	53.1	6	0	
	guts	281	93	188	66.9	39	13	4.6
	whole	7	3	4	57	2	0	
	Total	352	126	225			13	
Oregon	meat	28	12	16	57.1	2	0	
	guts	116	18	98	84.5	23	2	1.7
	whole	4	2	2	50	3	0	
	Total	148	32	116	32		2	

ND = None Detected.

TABLE 4.

Summary of the anchovy analyses between October 1991 and December 1992 for the California coastline.

County	# Analyses	# Positive	# >20 ppm	Range
Pacific	3	0	ND	ND
Humboldt	2	2	0	2.5-6
Alameda	6	0	0	ND
Santa Cruz	3	0	0	ND
Monterey	49	46	42	ND-2300
Los Angeles	1	1	0	Trace
Orange	8	6	2	ND-65
San Diego	5	3	1	ND-22

ND = None Detected.

### DISCUSSION

As indicated in the introduction the finding that the incident of shellfish poisoning that occurred in Canada several years ago was due to the presence of the toxin, domoic acid, in freshly harvested mussels, coupled to the dying seabird incident in Monterey Bay, prompted the initiation of seafood surveillance programs by both the FDA and the California Department of Health Safety. Early in FDA's program, analyst Gary Skow, from the Washington State Public Health Laboratory made a critical observation while conducting PSP analysis in razor clams using the mouse bioassay. He observed that mice used for the bioassay procedure exhibited symptoms that were suggestive of domoic acid poisoning (Iverson et al. 1989, Tryphonas et al. 1990) and informed the Seattle District FDA Laboratory of his findings. In response, the Seattle District established the presence of domoic acid in his samples by HPLC and based on these findings, initiated an expanded program of razor clam sampling. The geographic distribution of the samples collected and the results of the analyses are listed in Table 1. Of the 392 samples investigated, essentially all (98%) were found to contain domoic acid and a full 42% were found to contain domoic acid in excess of 20 ppm, a level considered to be unsafe for human consumption by the FDA. These results clearly signaled the presence of a major problem. Fortunately razor clam harvesting is primarily a recreational endeavor and the season had only been opened for a few days prior to being terminated by the Washington State Dept. of Fisheries and Wildlife as a consequence of

TABLE 5.

Monterey country anchovies, frozen, whole.

Sample #	Meat ppm	Guts ppm	Meat/guts Ratio	Harvest Date
1	23	170	0.135	11/1/91
2	47	410	0.11	11/6/91
3	50	370	0.135	11/10/91
4	25	350	0.07	11/10/91
5	74	380	0.19	11/13/91
6	71	290	0.24	11/16/91
7	47	2300	0.02	11/16/91
8	170	740	0.23	11/18/91
9	78	500	0.16	11/18/91
Total	585	5510		
Average	65	612	0.11	

the District's findings. As the levels of domoic acid diminished to acceptable levels (approx. 15 ppm), and, if in season, the beaches were reopened for harvesting. Since razor clams are confined to the northern coastal areas of California, their numbers are relatively small and commercial harvesting is a minor enterprise. Thus it is not surprising that a similar contamination problem failed to emerge. Regular periodic sampling of razor clams, as well as other clam species and mussels, together with phytoplankton are now being monitored by sample collectors for the Departments of Health of each of the Pacific Coast states. The monitoring program has been established to insure that if contamination reoccurs it will be detected early so that both the public and industry can be forewarned and poisonings avoided. Early on phytoplankton mon-

TABLE 6.

Imported seafood samples analyzed.\*

Composite	# Analyses	Product	Source
Tail	2	Lobster	Australia
Tail	1	Lobster, Rock, Frozen	Australia
Meat	2	Salmon, fresh	Canada
Meat	1	Mahi Mahi, fresh	Costa Rica
Meat	1	Tilapia	Costa Rica
Meat	1	Salmon, fresh	Faeroe Islands
Whole	4	Mackerel, canned	Fiji
Whole	4	Herring, canned	Germany
Whole	1	Anchovies, dried	Hong Kong
	3	Anchovies, canned	Italy
	1	Anchovy Paste	Italy
	1	Anchovies, Boiled Baby	Japan
Whole	4	Anchovies, dried	Japan
	1	Anchovies, white, frozen	Japan
	1	Anchovies, whole, dried	Japan
Whole	1	Anchovies, whole, frozen	Japan
Whole	8	Sardines, canned	Japan
Whole	1	Anchovies, dried	Korea
	1	Anchovy Sauce	Korea
Whole	1	Saury, Pacific, Salted	Korea
	1	Anchovies, canned	Malaysia
	1	Anchovies, dried	Malaysia
Whole	1	Anchovies, canned	Malaysia
Whole	2	Anchovies, canned	Morocco
Meat	2	Anchovy Fillets, canned	Morocco
Whole	1	Scampi, whole, frozen	New Zealand
Whole	8	Sardines, canned	Norway
Whole	1	Mackerel, striped, smoked	Philippines
	2	Sardines, canned	Philippines
Whole	1	Sardines, smoked	Philippines
	1	Shrimp Paste	Philippines
Whole	3	Sardines, canned	Portugal
	1	Anchovies, canned	Singapore
	3	Anchovy Fillets	Spain
Meat	2	Anchovy Fillets, canned	Spain
Meat	1	Pike Eel Fillet, canned	Taiwan
Meat	1	Mackerel, canned	Thailand
Whole	11	Sardines, canned	Thailand
Meat	1	Shrimp, Black, Tiger, raw, frozen	Thailand
Meat	1	Shrimp, canned	Thailand
Meat	1	Swordfish, frozen	Thailand

\* The specific species of the individual seafood samples analyzed is unknown.

itoring was recognized as having significant potential as an effective and efficient methodology for detecting domoic acid in the environment. As a consequence, all 3 western states began to employ phytoplankton monitoring on a routine basis. The expectations regarding the potential of phytoplankton monitoring have been largely realized, particularly by the early results from California. As a consequence, the monitoring program has been expanded and continues in all 3 states.

As indicated in the Results, samples of mussels and oysters from the Washington and Oregon coasts were collected along with the razor clam samples on the same days from the sites established by the National Shellfish Sanitation Program for monitoring PSP toxin. In sharp contrast to the razor clams, neither the mussels nor oysters showed any significant sign of domoic acid contamination. Why there should be such a dramatic difference between the species is not apparent but may be a consequence of faster rates of depuration and/or metabolism of domoic acid in mussels (Novaczek et al. 1992) and oysters relative to razor clams. However, the exact mechanism remains to be determined. These results have prompted public health authorities in Oregon and Washington, to use razor clams as a primary species signaling the environmental presence of domoic acid, from whatever source. The presence of the toxin in razor clams would be a clear signal that a domoic acid contamination had occurred and that other species need to be tested. During the period of this study (November 1991 through September 1993) a program monitoring mussels and oysters for the presence of domoic acid was also in place in California. Data supplied by the California Department of Health Services and the FDA, San Francisco District, indicates that similar results were realized, i.e., except for a few samples early in the program, trace levels of domoic acid were generally found. While razor clams are not an appropriate indicator species for California because of geographically non-uniform populations, anchovies, sardines and/or crab viscera may be, as discussed below.

Similar to razor clams, cooked crab appear to be highly susceptible to domoic acid contamination. As indicated in Results approximately 50% of all crab samples collected were found to contain some level of the toxin in the viscera irrespective of species or area of harvest. It is noteworthy, although perhaps not unexpected, that Dungeness crab viscera in general contains much higher levels of the toxin than the flesh. Indeed, the levels of toxin in the viscera was consistently 5 to 10 times higher than the level found in the meat (Table 5).

Of all the species investigated in this study, anchovies were found to be the most highly contaminated and therefore potentially pose the greatest risk to human health. As indicated in Results, not only did 75% of the samples investigated contain domoic acid but greater than 75% of the positives contained the toxin at levels in excess of the 20 ppm FDA action level. Indeed, several of the samples contained domoic acid at levels (in excess of 500 ppm, Table 4) comparable to the levels (up to 1000 ppm) found in mussels in the Prince Edward Island outbreak that resulted in incidents of brain damage and several fatalities. Clearly, anchovies is a species that needs to be monitored on a continuing basis and is, in fact, being monitored by the California Department of Health Services.

Of the 14 different species of imported samples tested for domoic acid, which included both lobster and anchovies, none was found to contain domoic acid even at trace levels (Table 6). However, since blooms of diatoms, specifically *Pseudonitzschia australis* (Garrison et al. 1992), are believed to be the source of domoic acid, seafood contamination would not be restricted to any specific geographic area but would be expected to arise wherever a bloom of the diatom occurs. Therefore, it is important to know whether blooms of the offending diatoms occurred either at the time or in areas of import harvests. If this information is not available it may be desirable to initiate an ongoing program for monitoring imported seafood for the presence of domoic acid or for restricting imports from countries that do not comply with ISSC biotoxin monitoring programs.

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**ABSTRACTS OF TECHNICAL PAPERS**

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**SERIAL SPAWNING OF THE GEODUCK CLAM (*PANOPAEA ABRUPTA*).** J. Harold Beattie, Washington Department of Fish and Wildlife, Point Whitney Laboratory, 1000 Point Whitney Road, Brinnon, WA, 98320.

Geoduck clams represent a valuable resource both in Washington State and British Columbia. The ex-vessel value of geoduck increased from \$.50 per pound to \$7.50 per pound from 1987 to the present. The Point Whitney Laboratory has been culturing geoduck since the early 1970's. The standard mode of operation for obtaining gametes was to bring adult animals to the hatchery, hold them for conditioning for 2 to 6 weeks and spawn them once. This entailed using hundreds of animals for each spawn, thousands of animals over the spawning season. Egg take per female was approximately 10 million. The current value of geoduck renders that method impractical; broodstock costs would be exorbitant. Using the same broodstock for more than one spawn would represent a more economical approach.

In 1994, we undertook a serial spawning experiment at Point Whitney. Our dive team collected 86 geoducks on 10 February 1994. These animals were held in flowing sea water at 10°C and received a supplemental cultured algal diet. On 17 March 1994 we attempted and succeeded with the first spawn. Each week thereafter we attempted to induce spawning with these same animals. The group spawned successfully nearly each week from 17 March through 13 July. The number of males spawning was always more than the number of females. The number of males that spawned each week varied between 4 and 44. The number of females that spawned varied from 0 to 29. The spawning of females appeared to be cyclical with more individuals spawning every second or third week. Total eggs spawned over the season was over 2.3 billion; one female spawned ten times.

**CAUSES OF MORTALITIES AMONG CULTURED JAPANESE SCALLOPS (*PATINOPECTEN YESSOENSIS*) IN BRITISH COLUMBIA.** S. M. Bower\* and G. R. Meyer, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

Mortalities that occur during grow-out of cultured Japanese scallops in British Columbia have been attributed to at least 4 agents. At one location, the shell boring polychaete, *Polydora websteri*, was responsible for 84% mortality in scallops approaching 5 cm in shell height. Most surviving scallops were stunted and regrowth of the shell was often abnormal. In the late summer of 1992, predation by numerous flatworms, *Pseudostylochus ostreophagus* (Class Turbellaria) caused high mortalities (up to 100%) among juvenile Japanese scallops (about 1 cm in shell height) being cultured at 2 localities. The third agent is an illusive protozoan of unknown taxonomic affinities (called SPX) that has sporadically occurred at several localities and caused mortalities approaching 100% in juveniles less than 3 cm in shell height. All ages of scallops were susceptible to infection but direct transmission between scallops occurred only when a flagellated stage was

produced in the tissues of about 15% of the infected, juvenile scallops. The identity of the fourth agent has not been established but it is thought to be an intracellular bacterium that infects haemocytes. Data suggest that this disease is wide-spread, chronic and induced by stress; it is usually associated with poor growth, shell deformities, persistent mortalities, and pinkish-orange pustules that are most evident in the adductor muscle.

**LIPID SPRAY BEADS FOR THE DELIVERY OF WATER-SOLUBLE MATERIALS TO MARINE BIVALVES.** Michael A. Buchal\* and Christopher J. Langdon, Oregon State University, Hatfield Marine Science Center, 2030 S. Marine Science Drive, Newport, OR 97365.

Little is known about the dietary requirements of marine bivalves for low molecular weight, water-soluble nutrients such as water-soluble vitamins and amino acids. Techniques for microencapsulating these materials for their controlled delivery to bivalves are limited by the small particle size required for ingestion ( $\leq 25 \mu\text{m}$ ) as well as by the digestive capability of bivalves. Spray cooling may be used to produce "spray beads" which encapsulate both aqueous and particulate materials in a matrix of lipid, such as the triglyceride tripalmitin.

Clam spat (average shell length 11.6 mm) were fed one of 3 types of lipid spray beads that either were stained with Sudan III, or contained a particulate core of antibiotic, or contained an aqueous core of polymeric dye. Softening tripalmitin bead walls with the addition of 40% w/w fish oil appeared to be necessary for digestion of beads. Softened spray beads present in clam feces were distorted and the remaining amount of core material was reduced. Counter-staining these spray beads in fecal samples suggested conversion of the triglyceride fraction of the bead wall to free fatty acids. Addition of fish oil to the spray bead wall, while increasing bead digestibility, reduced encapsulation and retention of aqueous core material, but had no effect on the encapsulation of particulate material.

These tests of digestibility and spray bead performance indicated that lipid spray beads may be effectively used for delivery of slightly soluble or insoluble nutrients to marine bivalves.

**PEARLS BY THE SEA-OYSTER FARMING IN THE SOUTH PACIFIC.** Daniel Cheney\*, Maria Haws, and Kelvin Passfield, RDA International Inc., Placerville, CA.

Pearls are an important feature of the emerging economies of the South Pacific. Almost every tropical Pacific nation is now looking to the cultured pearl as a means to boost trade and employment, particularly on the more remote islands. Pearl culture has all the factors common to farming of other shellfish, with the added twist of producing pearls.

The video presentation will show aspects of black-lipped pearly oyster (*Pinctada margaritifera*) spat collection, growout, seeding, and marketing. With emphasis on developments at Penrhyn (Tongareva) Atoll in the northern Cook Islands, the video will present

an early look at the pearl oyster hatchery, research laboratory and training center under construction on the island. The training center will support the approximately 200 small oyster farmers in the country, and will collaborate with other pearl producers, hatchery operators, and researchers in French Polynesia, Australia, and Hawaii.

**THE POTENTIAL INFLUENCE OF CORDGRASS *SPARTINA ALTERNIFLORA* ON CLAM RESOURCES IN WILLAPA BAY, WASHINGTON.** Brett R. Dumbauld\*, Martin Peoples, Lester Holcomb, Jack Tagart, Washington State Department of Fish and Wildlife, P.O. Box 190, Ocean Park, WA 98640, and Stephen Ratchford, School of Fisheries, University of Washington, Seattle, WA 98195.

The cordgrass *Spartina alterniflora*, a native salt marsh plant along the Gulf and mid-Atlantic coasts, was introduced into Willapa Bay, WA, with oyster spat in the late 1800s. It was recognized as a significant threat in 1987 after several successful years of seed set and an environmental impact statement was completed for control of the plant in 1992. Because it forms dense monotypic stands in the upper intertidal area and raises the elevation of the mudflat, we hypothesized that it could replace habitat and seriously affect the distribution of intertidal clams. A survey of adult clam distribution around *Spartina* clones was made in 1992 which revealed that hardshell clams, *Tapes philippinarum*, were actually more abundant just inside the perimeter of a clone than in the adjacent flat while the eastern softshell clam, *Mya arenaria*, displayed no such affect. Hardshell clams were significantly less abundant in the center of the clones while softshell clams were either not affected or more abundant within clones. Growth of hardshell clams was examined and found to be significantly reduced within clones. Separate studies on recruitment showed an apparent increase in settlement but subsequent decrease in survival along the inside perimeter of *Spartina* clones.

**DEVELOPMENTS IN SHELLFISH CULTURE IN BRITISH COLUMBIA.** William A. Heath, B.C. Ministry of Agriculture, Fisheries and Food, 2500 Cliffe Avenue, Courtenay, B.C. V9N 5M6.

Diversification of the B.C. shellfish industry is advancing with a number of new species culture initiatives underway. Progress on culture of scallops, geoducks, east coast mussels, and pinto abalone, as well as advances in offbottom oyster harvesting methods are described. Scallop developments include successful hybridization of Japanese (*Patinopecten yessoensis*) and weathervane (*P. caurinus*) scallops for disease challenge studies, improved seed production strategies and subtidal bottom sowing trials. Geoduck culture research is currently focusing on nursery and growout methods for commercial application. Growout trials are also proceeding for Atlantic mussel (*M. edulis*) hatchery seed to examine commercial feasibility for culture in B.C. Preliminary studies for

an abalone hatchery and growout facility are also underway. A mechanized harvesting system for offbottom cultured oysters has recently been tested and demonstrated.

**LIFE CYCLE, DISTRIBUTION AND LACK OF HOST SPECIFICITY OF *MIKROCYTOS MACKINI*, THE CAUSE OF DENMAN ISLAND DISEASE IN PACIFIC OYSTERS, *CRASSOSTREA GIGAS*.** D. Hervio\*, S. M. Bower, and G. R. Meyer, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

Denman Island disease, caused by the protozoan parasite *Mikrocytos mackini*, can account for significant losses to oyster growers. The prevalence of mortalities was estimated to be about 30% in some years on some beaches. Furthermore, it is the larger market sized Pacific oysters (*Crassostrea gigas*) that are infected, and the infected oysters that do not die have unsightly green pustules. Field and laboratory experiments indicated that *M. mackini* is directly transmitted between oysters during the spring (March to June) but the disease did not occur until the following spring in the newly exposed oysters. Recent investigations suggest that incubating exposed Pacific oysters at 18°C for about 3 months affected the development of the disease and significantly reduced the prevalence and intensity of infection. Conversely, the disease is lethal for exposed Pacific oysters incubated at about 10°C. This suggested that Denman Island disease could have a wider distribution and be a greater problem for other areas than the Strait of Georgia, British Columbia, where *M. mackini* is believed to be ubiquitous.

A field exposure trial conducted during the spring of 1993 confirmed the results of laboratory experiments that showed a lack of host specificity of *M. mackini*. All 4 species of oysters occurring in B.C. (the Pacific oyster *C. gigas*, the eastern oyster *Crassostrea virginica*, the Olympia oyster *Ostrea concaphila* and the European flat oyster *Ostrea edulis*) were susceptible to infection. These results indicate that precautions are essential to prevent the transmission of Denman Island disease from one species of oyster to another and that extreme caution is necessary when transplanting or moving the Pacific oyster and other species of oysters derived from areas where *M. mackini* is known to occur.

**THE HATCHERY PRODUCTION OF THE ALASKAN LITLENCK CLAM *PROTOHACA STAMINEA*.** Jeff Hetric\*, Carmen Young, and Maranda Barrier, Quteckak Shellfish Hatchery, P.O. Box 1467, Seward, AL 99664.

Adult Littleneck clams, *Protohaca staminea*, from Tatitlek, Prince William Sound, were transported to the Quteckak Shellfish Hatchery in Seward in January 1994. Four broodstock families of 15 individuals each were conditioned in mildly aerated 60 liter tanks at 16°-18°C and fed daily mixtures of *Tahitian isochrysis* and *Chaetoceros calcitrans*. Maturation was monitored biweekly by dissecting gonadal tissue. The first spawning event occurred, without inducement, on 22 February 1994. Spawning occurred throughout the summer at approximately 2-week intervals.

Larvae were cultured in 60 liter tanks. When the clam larvae reached approximately 240 $\mu$ m at day 26 they were transferred to an 130 liter airlift system on the downwelling mode. Larvae were fed a mixture of *T. iso*, *C. calcitrans* and *Thalassiosira pseudonana* twice a day at approximately 70,000 cells/mL per feeding. *Tetraselmis suecica* was added to the mix after the sixth spawn.

The temperature, setting substrate, feed ration, feed composition and frequency of changing out the water were altered with each cohort until the clams successfully metamorphosed after the sixth spawning. Successful setting occurred with a combination of fully mature gametes, 16°-18°C and 22 ppt filtered (2 $\mu$ m) seawater, 3-day cycle on changing the water and the addition of *T. suecica* to the algae mix. Presently, 4 cohorts (approximately 1,000,000) are under culture at 1-2 mm. Unusual preset and post set behavior has been observed.

**PHYSIOLOGICAL ALTERATIONS OF THE BLACK ABALONE, *HALIOTIS CRACHERODII* LEACH, WITH WITHERING SYNDROME.** G. Kisonhandaka, W. Roberts, R. P. Hedrick and C. S. Friedman\*, University of California, Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA, 94923.

Population densities of black abalone, *Haliotis cracherodii* Leach, have steadily declined on the California Channel Islands and numerous mainland locations in southern California beginning in 1985. Mortality has been attributed to a terminal disease called withering syndrome (WS) in which abalone become weak, lethargic and emaciated. The cause of WS has not been identified. We have initiated an investigation of physiological parameters, including oxygen consumption, ammonia excretion and food consumption, of black abalone with and without WS in order to identify which systems may be affected by this serious disease. Abalone were collected from locations where WS occurs and those where WS has not been observed during the summer and fall of 1992 and spring of 1993. All physiological parameters measured did not differ between sampling periods for each location sampled ( $p > 0.100$ ). Results suggested that weight specific oxygen consumption exists in abalone with and without WS ( $p = 0.000$ ) and that oxygen consumption rates did not differ between these 2 groups ( $p = 0.476$ ). Mass specific ammonia excretion was observed in abalone with WS in the summer and fall of 1992. During these times, ammonia excretion rates per gram body mass (wet and dry weight) were significantly different between healthy and sick black abalone ( $p < 0.001$ ). Although ammonia excretion rates were independent of weight in healthy ( $p = 0.059$ ) and diseased ( $p = 0.156$ ) in spring of 1993, excretion rates of abalone with WS tended to increase with increasing body mass. Rates of food consumption were not significantly different between abalone with and without WS ( $p > 0.100$ ,  $p = 0.107$ ). Microscopic examination of stained tissue sections revealed that abalone with WS deplete food muscle glycogen before muscle degeneration was observed. Severe foot muscle fiber depletion was consistently ob-

served in abalone in advanced stages of the disease. These data suggest that abalone with WS may be using foot muscle protein as an energy source.

**HYBRID VIGOR IN PACIFIC OYSTERS: AN EXPERIMENTAL APPROACH USING CROSSES AMONG INBRED LINES.** Daniel J. McGoldrick\* and Dennis Hedgcock, University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923.

In order to document and analyze the genetic basis of growth and survival in Pacific oysters (*Crassostrea gigas*), five 2  $\times$  2 crosses were conducted in the summer of 1993 and 2 in the summer of 1994. The outcomes of these experiments have substantial implications for genetic improvement because they demonstrate that cross-breeding can improve traits valuable to production. To confirm pedigrees and levels of inbreeding in the parents of each cross, allozyme markers were utilized. These markers detected contamination of broodstock in 3 of our 5 experiments. In 1 of the 1993 crosses there was significant hybrid vigor for survival and shell length. Hybrid vigor for shell length persisted and increased from larval to adult stages. In addition, reciprocal cross differences for shell length, which were statistically significant on days 7 and 14, disappeared by day 340, consistent with the action of a diminishing maternal effect. Density had no effect on larval shell length although larval density varied over 4 orders of magnitude. Therefore, factors independent of larval density and correlated to the parents must have increased the performance of the hybrid groups. This is consistent with previous observations of non-additive genetic variance in similar traits. In another experiment, hybrid larvae survived better than inbred larvae (thus exhibiting heterosis). Curiously, however, hybrids in this experiment were smaller than inbreds at both larval and juvenile stages. The two 1994 experiments await statistical analysis. Further studies using pairwise intercrosses of F1 hybrid groups, and molecular markers (microsatellites) can now be utilized to uncover and map the genetic basis of the demonstrated heterosis in Pacific oysters.

**SANITARY SURVEY AND COMMUNITY INVOLVEMENT A SUCCESS STORY.** Thomas Noland\*, Environmental Health Specialist II; Doris Robbins\*, Edison Sewage Committee Chairperson.

In the summer and fall of 1993 a sanitary survey was performed in the Samish Bay Watershed funded by a grant from the Centennial Clean Water Fund. The Communities of Edison and Blanchard in Skagit County WA were surveyed and a 55% failure rate was found in both communities. These failures were identified by using charcoal packets and sodium fluorescein dye. A slide presentation was developed showing the failures and what they look like. This presentation also included how different types of systems can be installed to repair identified failures. Both of these communities through education and presentations realized the po-

tential threat they posed to public health and the shellfish industry in the bay and are currently repairing these failures. Community involvement was very important and is presented with discussion on how to get the community involved in the repair of failing septic systems and sources for funding the repairs.

**MANDATORY HACCP PROGRAMS FOR PROCESSING MOLLUSCAN SHELLFISH.** W. Steven Otwell\* and Victor Garrido, Food Science and Human Nutrition Dept., University of Florida, Gainesville, FL 32611.

The Food and Drug Administration's proposed mandates for Hazard Analysis and Critical Control Point (HACCP) programs for the nation's seafood processors is progressing toward an expected final rule in summer 1995. All preliminary indications suggest molluscan shellfish processing, particularly for edible raw products, will be a focus for initial HACCP implementation. The recent Interstate Shellfish Sanitation Conference meeting (Aug. '94, Tacoma) advanced this speculation with a resolution to embrace HACCP and integrate necessary changes in the National Shellfish Sanitation Program's operational manuals. Anticipated changes include blending HACCP with the recently developed Plant Standardization inspection procedures. Anticipating this situation, a USDA funded project has installed HACCP programs in oyster processing firms in Florida and Louisiana. These pilot-efforts were structured to provide initial experience and critic by industry and responsible state inspectors. The HACCP plans and experience will be reviewed in comparison with potential applications to generic shellfish processing operations for cultured and natural harvested bivalves.

**PROPOSED REVISIONS TO WASHINGTON STATE SHELLFISH TRANSPORT REGULATIONS.** Robert E. Sizemore\*, Point Whitney Shellfish Laboratory, Washington Department of Fish and Wildlife, Brinnon, WA 98320.

Shellfish disease control regulations serve to protect both the shellfish industry's farmed stocks as well a natural stocks in Washington from the potentially serious effects of introduced infectious diseases. Recent experiences on the east coast of North America and in Europe show that introduced infectious disease can eliminate entire industries. The recommendations of a state-tribal-industry-research task force have been used as a basis for a more fully articulated shellfish disease control policy which effectively manages the risk of disease introduction.

The shellfish disease control regulations establish that it is unlawful for any person to import or transfer shellfish into Washington without first obtaining a state permit. This includes the import of any marine invertebrate into the state for aquacultural, research, or public display purposes, but excludes shellfish which

are market ready and intended for human consumption. For import into Washington from the west coast commerce region, the regulations define detailed requirements for disease free tissue certification of imported stock and health history for the geographic source area. Import is permitted contingent upon the absence of Class A (highest risk) shellfish disease. The regulations also contain procedures for invertebrate species which are not established in Washington or for established invertebrate species located outside of the west coast commerce region.

**INCREASING THE YIELD OF RED ABALONE THROUGH SUPPLEMENTAL FEEDING OF THE RED ALGA, RHODYMENIA PALMATA.** George A. Trevelyan\*, Raymond C. Fields and Frank R. Oakes, The Abalone Farm, Inc., P.O. Box 136, Cayucos, CA 93430.

The viability of commercial abalone farming depends to a great extent on the growth rates of the populations under culture. Due to the long production cycle, small incremental changes in mean growth rate can dramatically affect the profit margin. The red alga, *Rhododymenia palmata* (also known as dulse), has been identified as a good natural feed for red abalone. The purpose of this study was to determine if supplemental feeding with dulse increased the growth rate and yield of commercial scale, *Macrocystis* fed populations.

Two size classes (8 mm and 13 mm) of *Haliotis rufescens* (28,000 in all) were divided into 4 pairs of floating cages and were fed *Macrocystis* (*ad libitum*). One cage from each pair received weekly supplementation with dulse. After 4-6 months, the cages were harvested, and weight gain, shell growth, and survival were measured.

Dulse supplementation had no effect on survival, which averaged 91%. However, mean shell growth was 19% greater in the dulse supplemented cages than in the controls ( $p = 0.01$ ). In addition, the mean population weight gain of the dulse supplemented cages exceeded that of the controls by 28% ( $p = 0.002$ ). Thus, supplemental feeding with dulse significantly increased the growth rate and yield of commercial populations of juvenile red abalone.

**GROWTH AND LONGEVITY OF THE CRAB, CANCER OREGONENSIS.** Sylvia B. Yamada\*, B. C. Baldwin and H. Metcalf, Zoology Department, Oregon State University, Corvallis, OR 97331-2914.

*Cancer oregonensis* has the potential to be a major predator inside suspended oyster modules. In a previous study, in which juvenile crabs were introduced into oyster trays, we observed a 40% reduction in the survival of oysters. The present study focuses on the life cycle of this predator.

Growth and survival of two *C. oregonensis* cohorts were followed inside oyster trays on San Juan Island, WA. The first cohort settled from the plankton in June 1991 and attained a carapace width of 28-36 mm within a year, while the second cohort grew from 32 to 42 mm between August 1991 and June 1992. It thus appears that, under favorable conditions of food and shelter, *C. oregonensis* can attain its maximum size within 2 years.

Survival of the 1991 and 1990 cohorts of *C. oregonensis* from

August 1991 to June 1992 was 68% (N = 56) and 89% (N = 9), respectively. Females survived better than males, thus resulting in a skewed sex ratio in favor of females. Sixty-five percent of the 1991 and 100 percent of the 1990 females were gravid during February 1992. By August 1993, the last crab from the 1990 cohort died. These results, together with those obtained from individually caged crabs, suggest that maximum longevity for *C. oregonensis* is between 3 and 5 years.



**ABSTRACTS OF TECHNICAL PAPERS**

*Presented at the 15th Annual Meeting*

**MILFORD AQUACULTURE SEMINAR**

Milford, Connecticut

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**INTRODUCTION AND OVERVIEW 15TH MILFORD AQUACULTURE SEMINAR.** **Walter J. Blogoslawski**, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Avenue, Milford, Connecticut 06460.

In this 15th year of the Milford Aquaculture Seminar, we registered a record number of participants, reflecting the steady growth in interest in aquaculture over that period of time. This seminar has expanded from a handful of persons meeting to discuss specific disease problems to today's diverse group concerned with many different aspects of aquaculture industry and science.

The papers we discussed ranged from observations concerning juvenile oyster disease through discussions of oyster, scallop and clam projects. We heard of the use of grant monies to start or revitalize various aquaculture initiatives for the Northeast fishing industry. Salmon culture and polyculture of sea scallops projects were presented as well as economic and market studies used to determine the feasibility of aquaculture ventures. In addition, there were several interesting posters and a video tape presenting information on scallop biology and culture in Tasmania, larval settlement enhancement of the blue mussel in China, and causative agents of bivalve disease in the northeastern United States.

The participation of our 32 speakers and exhibitors is appreciated. I should also like to acknowledge the sponsors of this unique seminar which include the National Marine Fisheries Service, Woods Hole, MA and U.S. Department of Agriculture's Northeastern Regional Aquaculture Center, N. Dartmouth, MA. The interest and cooperation of all who attended made this meeting a vital forum for the timely dissemination of information on aquaculture. Audience attendance, comments and discussion enable rapid advances in the field, helping to elevate aquaculture to technical and applied levels greater than would be possible without this gathering. This year, nearly 150 of us came together to continue that valuable tradition, representing 12 states and the District of Columbia, 14 finfish and shellfish enterprises, 12 marine laboratories, and 16 universities.

**AN ECONOMIC ANALYSIS OF THE POTENTIAL FOR COMMERCIAL OYSTER DEPURATION IN NORTHWEST FLORIDA.** **Charles Adams**, University of Florida, P.O. Box 110240, Gainesville, FL 32611, **Rebecca Dunning**, Tidewater Research Station, 207 Research Station Road, Plymouth, NC 27932.

Food-borne illness associated with the consumption of raw shellfish is one possible factor that has depressed the general demand for oysters harvested in the northwest (Suwannee Sound) Florida region. Controlled purification (depuration) has been identified as one possible method of improving public confidence in oysters and thereby increasing sales. This economic analysis focuses on the costs of production, not on revenue generation and

profitability. The analysis determines the anticipated costs of depuration processing for 12 design options (strictly adhering to published FDA standards) with operating capacities from 30 to 498 bushels per week. Based on projected capital and operating costs, the expected premium for the depurated product, anticipated supplies of shellstock, and the cost of shellstock, commercial scale depuration is not an economically feasible method of processing oysters in northwest Florida.

**AN OVERVIEW OF THE EAST HAMPTON TOWN SHELLFISH HATCHERY AND RESEEDING PROGRAM.** **John Aldred**, Town of East Hampton, 159 Pantigo Road, East Hampton, NY 11937.

The East Hampton Town Shellfish Hatchery is a public facility located in Montauk, NY. Built in 1989–90 with seed money from New York State, it is operated by the Town for the enhancement of local shellfish stocks. By agreement, 10% of yearly production is made available to the state for regional distribution. The desire for a facility of this kind was accentuated by the closure of the local striped bass fishery in 1984 and the virtual elimination of the bay scallop from the region due to brown tide in the years following 1985. These 2 species historically represented the largest earning potential for local inshore commercial fishermen.

The hatchery annually produces in the neighborhood of 10 million hard clam, oyster and bay scallop seed suitable for distribution onto public bottom. A 7,000-sq. ft. former U.S. Navy warehouse on Fort Pond Bay houses static water larval and pediveliger rearing systems, a 60-unit flowing water upwelling nursery, temperature controlled as well as mass culture algal systems, shop, lab, and office space. A field nursery in Napeague Harbor, Amagansett contains rafted tray and pearl net systems for final grow out to planting size. A second 72-unit upwelling nursery is in the process of being created in Three Mile Harbor, East Hampton to take advantage of warmer harbor waters and to provide alternatives in the eventuality of unanticipated water quality problems in one or another site.

Along with Town officials, hatchery personnel have also organized oyster relays from uncertified waters to provide harvest potential and participated in designating management areas to protect spawning stocks. Projects being planned include a demonstration of bay scallop spat collection techniques for the Peconic estuary, a pilot oyster aquaculture project for local fishermen and an overwinter survival study of hard clam seed in different sediment types.

**THE GROWTH OF SALMON CULTURE.** **James L. Anderson**, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881.

The aquaculture of salmon has become one of the most significant influences in the salmon industry. Pen-raised salmon aquaculture has moved from virtual nonexistence in the late 1970s to

comprising over 30% of the global harvest in the 1990s, and is still growing. Aquaculture in the form of salmon enhancement or salmon ranching has also become the dominant source of "wild" salmon in many areas of the world, such as Japanese chum runs, the Columbia River, and pink runs in Prince William Sound, AK.

Aquaculture of salmon has influenced profound changes in the marketing and trade of salmon, as well as salmon fisheries management. This presentation will attempt to document the importance of salmon aquaculture, its increase over the past 2 decades, and how it has changed the salmon industry worldwide.

**SEASONAL PREVALENCE OF GONADAL NEOPLASMS IN CLAMS, *MYA ARENARIA*, IN MAINE AND IMPACT ON OOGENESIS.** Bruce J. Barber, Department of Animal, Veterinary & Aquatic Sciences, University of Maine, Orono, ME 04469.

A preliminary survey of several clam populations in Maine in 1993 revealed a 42% prevalence of gonadal neoplasia in Bells Cove, Whiting Bay. This population was sampled further in 1994 to determine seasonal patterns in both prevalence and intensity and also to quantify the effects of this disease on gametogenesis.

Thirty adult clams were collected in all months but February, fixed in Helly's fixative and stained with hematoxylin and eosin for histological examination. Each clam having a gonadal neoplasm was assigned an intensity level of either Stage 1 (<50% of gonadal follicles containing neoplastic cells); Stage 2 (>50% of gonadal follicles containing neoplastic cells); or Stage 3 (metastasis evident). An image analysis system was used to examine 5 fields of gonadal tissue from each female clam and to determine mean egg (oogonia, oocyte, ova) number per field and mean diameter of those cells present.

Prevalence in 1994 ranged from 10% in June to 27% in September and October. Mean intensity, on a scale of 0–3, was maximal in April and May, with a value of 2.1. Increases in prevalence and intensity from January through May, followed by decreases in June, suggest that the disease was progressive and caused mortality after May, during the period of maximal gametogenic activity. Mean egg number per field was significantly ( $P \leq 0.05$ ) lower in diseased clams than in healthy clams. Mean egg diameter of diseased clams was significantly ( $P \leq 0.05$ ) lower in diseased clams than in healthy clams from January through June (before spawning), but significantly ( $P \leq 0.05$ ) greater in diseased clams than healthy clams from August through December (after spawning).

The reduction in egg number in diseased clams is strictly a function of the relative number of follicles that contain neoplastic rather than normal cells. The lower egg diameter before spawning and greater egg diameter after spawning in diseased clams relative to normal clams is the result of a general inhibition of the normal gametogenic process, and occurs throughout the entire gonad, not just affected follicles. Thus the impact of neoplasia on gametogenesis at the individual level can be extensive. Given a high enough

prevalence, this disease could also negatively impact reproductive output at the populational level.

**MSX LIFE CYCLE: SPORES AND NON-OYSTER HOSTS.** Robert D. Barber and Susan E. Ford, Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 587, Port Norris, NJ 08349.

The life cycle of *Haplosporidium nelsoni*, the causative agent of MSX disease in eastern oysters, is unknown, but an intermediate or alternate host has long been suspected. Although sporadic searches for *H. nelsoni* in other organisms have been conducted since the parasite was first discovered, they have not been systematic and thus have rarely yielded concrete results.

We have conducted a focused, methodical 2-year search for an alternate or intermediate host for *H. nelsoni* in lower Delaware Bay where the parasite is usually abundant. Alternate hosts are typically similar to the definitive or normal host and have similar parasite life stages. Intermediate hosts, on the other hand, are usually dissimilar to the normal host and parasite forms in them can also be very different. One stage in the *H. nelsoni* life cycle, the spore, is extremely rare in adult oysters but fairly common in spat and yearlings. Because small oysters seem to support the complete life cycle (i.e., spores are produced), we reasoned that an alternate host would be similar. Consequently, we regularly collected specimens of several small bivalve species for examination as potential alternate hosts. In choosing possible intermediate hosts, we focused on zooplankton, a motile group that could act as a dispersal mechanism for the parasite. We assumed that the presence and abundance of parasites in non-oyster hosts would be linked with their appearance and abundance in oysters. Thus, collection dates and places were selected based on knowledge of the spatial and temporal distribution of *H. nelsoni* infections in oysters.

Over the 2-year study, thousands of specimens of potential non-oyster hosts have been examined by histological section. To date, no parasites resembling *H. nelsoni* have been found. In fact, almost no protozoans have been identified in any of the small bivalves or zooplankters. Our study was designed to be thorough enough to exclude certain species as alternate or intermediate hosts if we did not find *H. nelsoni* in them. During the study period, however, levels of *H. nelsoni* in Delaware Bay oysters of all ages were unusually low. We cannot therefore exclude the possibility that a non-oyster host was among the species examined, but that the low *H. nelsoni* prevalence during the study (as measured in oysters) precluded finding the parasite in this host. Given the large number of specimens examined, however, we think we should have found at least a few individuals containing suspect parasites if an alternate or intermediate host had been among the species examined. Certainly, another host could exist outside the groups examined. Alternatively, if such a host exists and is a small bivalve or a zooplankter, it may have been scarce or absent during the study period, which would also explain the low *H. nelsoni*

prevalence in oysters. Finally, the life cycle of *H. nelsoni* may be direct, with spat as the source of infective stages (spores), and not involve a non-oyster host. NJAES Publication #K-32405-2-95.

**THE FISHING INDUSTRY GRANTS PROGRAM: SPOTLIGHT ON AQUACULTURE.** **Kenneth Beal**, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, One Blackburn Drive, Gloucester, MA 02543.

In March of 1994, the Secretary of Commerce announced the availability of \$30 million under provisions of the Emergency Supplemental Appropriations Act of 1994 to address the needs of those directly affected by the decline of the traditional fisheries in the Northeast. Designated as the Northeast Fisheries Assistance Program, this initiative includes \$9 million administered by the National Marine Fisheries Service for Fishing Industry Grants (FIG). Under the first part of the FIG Program, \$2 million has been targeted for 9 aquaculture investigations to help restore overfished New England groundfish and shellfish stocks through hatchery programs, as well as to provide new business opportunities for displaced fishermen.

Aquaculture activities will focus on cod, haddock, summer flounder, Nori (*Porphyra*), quahogs, and scallops. The associated research will be conducted in various coastal locations in Maine, New Hampshire, Massachusetts, and Rhode Island. The work funded under the program responds to NOAA and NMFS strategic priorities for accelerating the growth of U.S. marine aquaculture.

**SUMMER FLOUNDER CULTURE: AN UPDATE ON RESEARCH AND THE DEVELOPMENT OF AN INDUSTRY.** **David Bengtson**, University of Rhode Island, Department of Zoology, Kingston, RI 02881, **George Nardi**, New England Fisheries Development Association, Inc., 451 D Street, Boston, MA 02210.

At the 1994 Milford Aquaculture Seminar, the results of a 3-year Saltonstall-Kennedy funded research program on summer flounder culture were presented. Since then, the levels of both interest in and research on this species have increased. Currently, the Saltonstall-Kennedy program is funding 2 projects. One is led by the New England Fisheries Development Association, with participation by the Universities of Rhode Island and New Hampshire and two private companies, GreatBay Aquafarms and Northeast Organics. This project continues research from the first S-K project and particularly investigates pigmentation abnormalities, cannibalism and weaning at the time of metamorphosis, maximum stocking density for grow-out in recirculation systems, and the economics of summer flounder culture. The second S-K project, at North Carolina State University, primarily investigates the reproductive biology of summer flounder. In addition, the University of Connecticut is developing a summer flounder research program in conjunction with the Caribbean Marine Research Center.

The National Marine Fisheries Service, through its Northeast Fishing Industry Grants Program, is contributing to the development of the industry. An NFIG grant to AquaFuture, Inc. will bring about both a hatchery industry (joint venture of AquaFuture and GreatBay Aquafarms) and a grow-out industry employing those currently in the fishing industry (V&G Sea Products and Provincetown Select Seafoods). A second company, Mariculture Technologies, Inc. is independently establishing a summer flounder facility in eastern Long Island. A great deal of interest clearly exists in the future of a summer flounder culture industry.

**TOXIC ALGAE BLOOMS AND THEIR IMPACT ON SHELLFISH MARKETS.** **Priscilla Brooks**, Conservation Law Foundation, 62 Summer Street, Boston, MA 02110, **Cathy R. Wessells** and **Christopher J. Miller**, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881.

Toxic algae blooms are worldwide phenomena which appear to be increasing in frequency and severity. These naturally occurring events can generate substantial economic impacts including supply interruptions due to closed fishing grounds, losses from human illness and losses due to decreases in demand for both affected and unaffected seafood products.

Using selected North American algae bloom incidents as case studies, this presentation examines the demand side impacts which arise following public announcements and media reports of the dangers of consuming seafood harvested from affected areas. Public reports that certain seafood products from particular areas are toxic and should not be consumed create consumer fear and avoidance of those products which can then spread to other unaffected products. The result is typically a short-term and possibly long-term decline in demand for both contaminated and even uncontaminated seafood products.

**PERKINSUS PREVALENCE IN OYSTERS FROM LONG ISLAND SOUND: AN UPDATE.** **Diane J. Brousseau**, Biology Department, Fairfield University, Fairfield, CT 06430.

Prevalence rates for *Perkinsus marinus* were determined for 3 intertidal populations of *Crassostrea virginica* from Long Island Sound (Black Rock Harbor, Bridgeport, CT; Saugatuck River, Westport, CT; Milford Point, Milford, CT) from September 1993 to December 1994. The Ray thioglycollate assay was used for *P. marinus* diagnosis. Temperature and salinity data were collected for one site (Black Rock Harbor).

A seasonal cycle of prevalence was found in all populations studied. The highest prevalence rates occurred in the summer and fall with declining numbers of infected animals detected during the winter and spring. Prevalence rates were highest in the oysters from Bridgeport (20–100%) in nearly every month sampled. Oysters from Milford Point had the lowest monthly prevalence rates (<50%) of the 3 sites under study. The majority of the infections at all sites were classified as light (0.5–1.0, Mackin's scale), but

heavy parasite burdens were reported in some individuals from the Bridgeport and Westport populations.

Water temperatures were favorable for parasite proliferation from mid-June to September and salinities were above 15 ppt throughout the study period. In spite of the record cold temperatures recorded during the winter of 1993–1994, *P. marinus* persisted in oysters from the Bridgeport and Westport populations. A group of experimental animals collected in March 1994 and held in a recirculating seawater table at 20°C for 5 weeks showed a 3-fold increase in parasite prevalence during the study period. It is not known whether this result was due to the stimulation of sub-patent infections or the appearance of new infections in the oysters under study.

**CULTURED AND NATURAL *PERKINSUS MARINUS* CELLS: A POSSIBLE MECHANISM FOR VIRULENCE DIFFERENCES.** Marnita M. Chintala, Kathryn A. Alcox, and Susan E. Ford, Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 587, Port Norris, NJ 08349, David Bushek, Belle Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Georgetown, SC 29442.

The recent development of methods for the *in vitro* propagation of the oyster pathogen *Perkinsus marinus* has greatly expanded the kinds of research that can be done on this parasite. A number of investigators, however, have found that cultured parasites appear to be less infective and pathogenic than those isolated directly from infected oysters. Loss of virulence among cultured parasites is common, although the mechanisms involved are not well understood.

To test for loss of pathogenicity in cultured *P. marinus*, we challenged oysters with both cultured and natural parasites. We monitored mortality rates for 12 weeks post-challenge, determined total parasite burdens in all oysters (gapers and survivors), and also counted parasites shed in feces and pseudofeces during the first two days after challenge.

Seventy-five percent of the oysters challenged with natural parasites died during the experiment and nearly all were heavily infected with *P. marinus*. Only 7.5% of the oysters exposed to cultured cells, and no control oysters, died. Although all oysters were challenged with equal numbers of *P. marinus*, the number of parasites recovered in feces and pseudofeces during the first 2 days after challenge was highly dependent on parasite source. Nearly 50 times more cultured parasites than natural parasites were voided in pseudofeces. In feces, the ratio of cultured to natural cells was 10 to 1. When feces and pseudofeces were combined, cultured parasites were expelled nearly 20 times more frequently than natural parasites.

The difference in mortality rates clearly shows that natural parasites were more virulent than cultured cells. Differential rejection rates suggest that oysters responded differently to parasites depending on whether they were natural or cultured. This was

especially true at the level of the gills and palps, where particles are sorted before entering the digestive system. The surface of wild cells may have receptors or other characteristics, which are reduced or lacking in cultured cells, that favor retention by the oyster. The number of parasites rejected in our experiment was a relatively small fraction of the total dose, however, and there are probably additional mechanisms (perhaps involving the internal defense system) that contributed to the observed virulence differences. NJAES Publication #K-32405-1-95.

**OBSERVATIONS ON SELF-FERTILIZATION IN THE BAY SCALLOP *ARGOPECTEN IRRADIANS*.** Joseph Chormanski and Sheila Stiles, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Avenue, Milford, CT 06460.

Spawning trials on the self-fertilization of bay scallops, *Argopecten irradians*, have led to the development of several inbred lines. The trials were initiated to measure the potential for producing homozygous scallops with outstanding characteristics, such as fast growth or increased muscle yield. Inbreds could be selected within lines or families for particular traits, then crossbred for heterosis. Inbred scallops also can be developed to evaluate environmental conditions, as they are more genetically uniform, and therefore should demonstrate a more striking response to external changes. For the present study, scallops were selected based on shell color or depth to use for inbreeding by self-fertilization. For example, the parent scallop for one inbred line had a striking yellowish-orange shell; most of its progeny had shells that were also yellowish-orange. This line seems to be quite robust for growth and survival. Another scallop that was self-fertilized had shells with 3–5 broad white stripes and the majority of its progeny had stripes that ranged from 1 to 5 with varying widths. This line appears to have somewhat lower viability. Generally, in contrast to mass-spawned cultures with survival to metamorphosis in 4 out of 4 crosses, only 4 of 8 self-fertilized cultures had larvae that survived to metamorphosis. In addition, development to 48 hours was significantly lower, and growth was retarded in the inbred scallop cultures, suggesting inbreeding depression. Preliminary results indicate the following: 1) scallops manifest inbreeding depression in early stages by decreased survival to the larval stage and to metamorphosis, and by retarded growth of larvae and early juveniles; 2) shell marks seem to be reflective of genotypes with a significant genetic component; 3) different inbred lines will have different degrees of fitness. The bay scallop, a functional hermaphrodite, provides an interesting model for genetic studies of bivalves to rapidly develop inbred lines through self-fertilization.

**DEMONSTRATION LONG-LINE SCALLOP FARM.** Thomas Cunningham, Bridgeport Regional Vocational Aquaculture

School, 60 St. Stephens Road, Bridgeport, CT 06605, **Luning Sun**, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China.

The Chinese Academy of Sciences (Academia Sinica) of the People's Republic of China and the Bridgeport Regional Vocational Aquaculture School are cooperating in grow-out studies of bay scallops (*Argopecten irradians*) in western Long Island Sound with help from a grant from the State of Connecticut's Department of Environmental Protection License Plate Program. With the invaluable help from staff of the National Marine Fisheries Service in Milford and Dr. Lance Stewart from the University of Connecticut, this project involves the development of various mass cultures of phytoplankton, the acquisition of native scallops, their spawning in the aquaculture school's laboratory and their deployment into the traditional Chinese lantern nets in a small demonstration long-line scallop farm to be placed in the waters off of Bridgeport. Various additional experiments are planned that include type of algal diets, comparative studies on various deployment sites and off-bottom culture techniques as well as the possible environmental impacts.

**COMPARISON OF SUSCEPTIBILITY OF GENETICALLY SELECTED LINES OF OYSTERS TO JUVENILE OYSTER DISEASE (JOD).** **Christopher V. Davis**, Darling Marine Center, Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Walpole, ME 04573, **Bruce J. Barber** and **Robert O. Hawes**, Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469.

Aquaculturists in Maine and northeastern United States have experienced high mortalities (>90%) of hatchery-reared juvenile eastern oysters (*Crassostrea virginica*) since 1988 which has raised serious concerns about the viability of hatchery-reared oyster farming in the stricken areas. Recent work has shown that the incidence of mortality is clearly size-dependent; oysters 15–25 mm shell height exhibit greater JOD-induced mortality than do larger seed oysters. This study explored the possibility that fast growing genetic lines may be less susceptible to JOD than comparable control lines as a result of selection for superior growth.

Broodstock from an F<sub>1</sub> "Flower" line of selected and unselected (control) sublines were spawned in March 1994. Selection of the parental lines was based on whole weight at 18 months of age. Larvae and spat were reared identically and deployed in replicate floating trays at 2 growout sites in the upper and lower Damariscotta River, ME. Growth, mortality and general health were monitored weekly from 13 July to 24 October at both sites. Timing and levels of cumulative JOD-induced mortality were correlated to both genetic group and growout site. Oysters in the select group grew faster and had a delayed and lower rate of mortality than the control group within each location. Furthermore, oysters at the lower (cooler, more saline) site showed a 3–4 week delay in mortality compared to the upper site. This work suggests that genetic selection for growth and disease resistance

may complement new management strategies to combat the effects of this disease.

**JUVENILE OYSTER DISEASE STUDIES 1994: EPIZOOTIOLOGY, GEOGRAPHIC OCCURRENCE.** **C. Austin Farley** and **Earl J. Lewis**, Oxford Field Station, Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA, 904 S. Morris Street, Oxford, MD 21654–9724.

Samples of cultured and feral juvenile oysters from New England and mid-Atlantic sites have been examined periodically for the last 3 years for the presence of juvenile oyster disease (JOD). Samples of oysters  $\geq 5$  mm ( $n = \geq 100$ ) were measured and mortality calculated on the basis of ratio of live oysters vs. intact valves and/or gaping dead oysters. Prevalence and size of shell checks and internal conchiolinous shell lesions in both live and dead oysters were also determined. This year, samples were examined from Oyster Bay, Peconic Bay, and Fishers Island, NY, and Rhode Island, Maine, and Maryland.

Seed oysters from Maine (presumed to be infected) were introduced by growers into Peconic Bay, Fishers Island, RI, and a number of sites in Maryland, including the hatchery at Deal Island. Varying evidence of JOD was seen in all of these locations. A 1994 introduction to Peconic Bay had 60% mortality and 77% conchiolin. In Rhode Island, there was 40% mortality and 73% conchiolin. At Fishers Island, no disease was seen in pond-reared seed <1 year old, but >1 year seed held in lantern nets adjacent to Fishers Island had 41% shell checks at 22 mm, 8% mortality, and 67% conchiolin. Rhode Island oysters had 63% mortality with 55% conchiolin and Maine oysters 33% mortality with 71% conchiolin. Maryland introductions showed reduced effects, probably due to decreased salinities. Seed introduced into Maryland in July 1993 showed significant mortality in September in at least one location. Survivors showed characteristic shell checks (up to 34% at 14 mm) in February 1994. A sample of these oysters held in 26 ppt salinity for 2 months experienced 79% mortality and exhibited 46% conchiolinous lesions characteristic of JOD, suggesting that (1) these oysters were already infected when introduced at 1 mm size, and (2) the pathogen was carried subclinically for 10 months until conditions conducive to manifestation of the disease occurred. Native seed produced in May 1994 at the Deal Island hatchery showed 79% shell checks at 13 mm in October but no mortality even after being held for 3 months at 26 ppt.

Samples of seed oysters produced in February, March, May, and June at the Flower and Sons hatchery in Oyster Bay had mortalities of 0–9% by 1 August, while the runts (14 mm) had 25% mortality. All groups showed significant shell checks (up to 42% at 12 mm). Mortalities in seed produced at Cedar Beach in Peconic Bay were 60% with 70% conchiolin.

In summary, juvenile oyster mortalities continue to occur at sites where previous problems existed. In situations where salinities are high, seed oysters from Maine experience typical mortalities. Chesapeake Bay seems to have been spared, even though

infected seed were introduced. Flower's hatchery mortalities were much lower this year due to a variety of factors.

#### STUDIES OF RESISTANCE IN PROGENY OF BROOD STOCK SELECTED FROM JUVENILE OYSTER DISEASE (JOD) SURVIVORS.

C. Austin Farley and Earl J. Lewis, Oxford Field Station, Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA, 904 S. Morris Street, Oxford, MD 21654-9724, David Relyea and Joseph Zahtila, Frank M. Flower & Sons Inc., P.O. Box 1436, Bayville, NY 11709.

Mortalities of juvenile oysters in the nursery trays at the Flower hatchery in Oyster Bay in 1990 were 70 to 90%. In 1992, brood stocks from a Connecticut native population (presumably uninfected) were used on the rationale that vertical infection from infected brood stocks from Oyster Bay could be avoided. Mortalities in oysters spawned after March 1992 continued at the 70–90% level.

Following our suggestion, brood stocks for 1993 progeny were selected from Flower's oysters that had survived the disease and demonstrated the characteristic primary, secondary, and tertiary shell checks at 12–15 mm, 20–25 mm, and 35–45 mm indicative of the disease. All progeny in 1993 were produced from this brood stock. Mortalities in April and June spawnings peaked at <40% (populations spawned prior to April had shown excellent survival in previous years and this strategy continued to be an effective management tool against JOD). In 1994, the same brood stock (Flower's survivors) were used to produce the commercial FMF-F<sub>1</sub> progeny. In addition, we suggested using a Connecticut brood stock naive to JOD to produce susceptible progeny (FCT-F<sub>1</sub>) as a control to study the development of possible resistance to JOD. Progeny from both brood stocks were produced in a second June spawning (JUN2). Duplicate populations of 2000 oysters from each F<sub>1</sub> batch were placed in three 4000-gal tanks which receive periodic replenishment from natural water: (1) unfiltered, (2) filtered at 50 µm, and (3) filtered at 25 µm. Additionally, oysters from each F<sub>1</sub> generation were placed in trays outside in the Flower hatchery racks and at the Suffolk County Marine Environmental Learning Center (SCMELC) at Cedar Beach, Peconic Bay.

After 10 weeks, FMF-F<sub>1</sub> in tank exposures showed <10% mortality; raft-exposed FMF-F<sub>1</sub> had <35% mortality and SCMELC-exposed, 45%. The tank-exposed FCT-F<sub>1</sub> had 80, 42, and 30% mortality, respectively; the raft-held FCT-F<sub>1</sub> showed 70% and the SCMELC-exposed FCT-F<sub>1</sub>, 55%.

This study clearly demonstrates that the F<sub>1</sub> progeny from selected surviving brood stock is up to 7 times as resistant to JOD as progeny of naive brood stocks. A factor that was noticed in histologic material of survivors was the presence of thickened mantle epithelia which contained unusual deposits of melanin. Also observed this past summer was FMF-F<sub>1</sub> seed with increased shell pigment which started abruptly at just beyond the first shell check at about 15 mm. The participation of melanization as a defense

mechanism in invertebrates is well known and operates as a cascade, resulting in the production of peroxidases that have lytic activity on foreign proteins of parasites and bacteria. This response may indeed be the mechanism of resistance in the disease.

#### POLY CULTURE OF SEA SCALLOPS SUSPENDED FROM SALMON NET PENS.

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The goal of this project is to determine the commercial feasibility of polyculture for sea scallops (*Placopecten magellanicus*) in suspension culture adjacent to salmon net pens in Maine. The farm-raised salmon industry has experienced dramatic increases in production in recent years, constituting a significant industry in coastal Maine. Super chilling of the lower Bay of Fundy caused massive fish mortalities, and increased production from other salmon producing countries coupled with large wild harvests have resulted in diminishing profit margins. As profit margins become increasingly small, diversification of species cultured is prudent for the financial stability of the aquaculture industry. Having an established facility, a platform on site and available labor, salmon farming operations are well suited for polyculture. Approximately 10,000 scallop seed collected from wild spat have been placed in pearl nets and suspended from the cages and mooring cables of net pens. Five hundred scallops have also been placed in modified lobster cages on the bottom at each site to serve as controls. Scallops are being sampled intermittently for shell height, volumetric measurements and adductor muscle weight to determine growth rates. Water temperature, turbidity, and flow rates are being monitored. Shellfish samples will be taken periodically to test for the presence of marine biotoxins, specifically Paralytic Shellfish Poison (PSP). The market for 6–8 gram adductor meats will be evaluated. In addition, the cost of grow out of 40 to 50 meat count scallops will be analyzed. The practicality of a polyculture operation through various conflicts with space, equipment, labor and the harvesting of multiple species will be determined.

#### HARD CLAM (*MERCENARIA MERCENARIA*) AQUACULTURE UNDER PRODUCTION UNCERTAINTY.

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Using a hard clam growth function for each of 2 different clam planting densities, hard clam growth was simulated for 10 and 15 mm seed planted at 62.5 and 75 clams per square foot. A 34-month growing period was selected for simulation purposes. Assuming that one could choose to plant seed during any given month, separate growth simulations for each of the 12 calendar months were conducted. Growth simulation results were then combined with mortality assumptions, production restrictions

(lease size and planting capacity) and size-dependent price expectations to estimate expected returns. These returns are then used to determine the best production method (seed size and planting density), planting and replacement schedule (growout times and replanting schedules) and maximum returns. The expected returns were then used to estimate the net returns and net present value of the lease. Production results were simulated for three hypothetical lease sites using environmental data from three locations in the Indian River Lagoon, FL, region.

Results indicate that, where possible, growers should purchase larger seed and plant at the higher density and that, regardless of production method used, plant and harvest scheduling requires special attention. Growout times, replacement schedules and expected revenues vary by site location as determined by the specific environmental conditions of each site, thus emphasizing the importance of site selection. Comparison of the economic results of this study with other studies that assume fixed growth and production conditions indicate that returns can be increased through adjusting production design to accommodate variable growth.

**WHAT DOES CONDITION INDEX TELL US ABOUT SOFTSHELL CLAM PHYSIOLOGY?** Dale F. Leavitt, Judith McDowell Capuzzo, and Bruce A. Lancaster, The McDowell Laboratory, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Condition index is a frequency measured parameter used to assess and compare bivalve physiological status. For example, bivalve condition index measurements have been used in studies of contaminant effects, geographical variability of physiology, nutritional physiology, and aquaculture protocols. Usually calculated as a ratio of the soft tissue component to the shell component of a bivalve multiplied by some constant, it is used as an overall indicator of the physiological condition of the animal. But what exactly does a condition index data point tell us about the bivalve's physiology? To answer this question, we sampled field-collected softshell clams (*Mya arenaria*) on a monthly basis for 18 months from 2 sites within Buzzards Bay, MA. The sites were chosen to ensure significant variations in condition index of the clams between sites over the annual cycle. The clams were analyzed for a large suite of morphometric, biochemical, and reproductive parameters including: valve length, live weight, soft tissue wet and dry weights, lipid, protein, and glycogen content of viscera and digestive-gonad complex, sex, and gametogenic status. These data were then statistically analyzed and compared, using multivariate techniques, to assess those specific measurements of importance in defining the condition index of the bivalve and what method of calculating condition index gave us the most information on the clam's physiology. The results of statistical analyses will be presented in the context of evaluating a clam's physiological status relative to experimental or routine aquaculture conditions.

**CONTINUING STUDIES OF THE ROLE OF BACTERIA (VIBRIO SPP.) IN UNEXPLAINED JUVENILE OYSTER MORTALITIES.** Mijin Lee, Gordon T. Taylor, and Monica Bricelj, Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794–5000, Susan E. Ford, Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 587, Port Norris, NJ 08349.

Juvenile oysters, *Crassostrea virginica*, cultured in the northeastern USA have experienced episodic mass mortalities, so called Juvenile Oyster Disease (JOD). Histopathology revealed that the symptoms were similar to other *Vibrio*-induced disease like Brown Ring Disease of the Manila clam in France. During the summer of 1994, healthy oyster seed stocks were subjected to challenge experiments with *Vibrios* isolated from infected oysters collected from our 1993 field study at the Frank M. Flower and Sons Oyster Hatchery. The first experiment was run under low stress conditions for 30 days, i.e., single inoculation, 22°C, crowded, detritus removed, and 2 daily feedings. The second experiment was run under moderate stress conditions for 14 days, i.e., 2 inoculations, 24°C, crowded, detritus not removed, and 1 daily feeding supplemented every other day with *Vibrio*. Oysters injected with 2 of 7 isolates experienced mortality significantly higher than controls (*E. coli* injected, filtered sea water injected, or no injection) or those injected with other isolates. Biochemically identical species of *Vibrio* were reisolated from the experimentally infected oysters and were the numerically dominant *Vibrios* in oyster tissue, strongly suggesting a link between infection by a *Vibrio* strain and JOD.

**TRANSMISSION AND FILTRATION STUDIES OF JUVENILE OYSTER DISEASE (JOD).** Earl J. Lewis and C. Austin Farley, Oxford Field Station, Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA, 904 S. Morris Street, Oxford, MD 21654–9724, David Relyea and Joe Zahtila, Frank M. Flower & Sons, Inc., Bayville, NY 11709, Gregg Rivara, Cornell University Cooperative Extension, Southold, NY 11971.

Since juvenile oyster disease (JOD) was first reported in the late 1980's, much has been learned about the disease syndrome from experimental and field studies. Although questions remain concerning the specific etiology of JOD, scientific and industry studies have led to the implementation of management techniques that have greatly improved the survival of exposed oysters at one New York oyster hatchery. Our 1994 experimental work focused on follow-up transmission studies in salinity controlled aquaria, field and aquaria studies using filtered JOD-infected water to better understand the size of the disease agent, and whether infected oysters are a necessary part of the transmission process. Further attempts to isolate bacteria from experimentally infected oysters were also made.

Salinity controlled, JOD-infected aquaria left dormant for approximately 8 months were used for additional transmission experiments. No mortalities were observed in susceptible juveniles after 6 weeks of exposure. Mortalities began during week 9 of the exposure in oysters held at salinities of 18–30 ppt, after JOD-infected oysters were added to each aquarium during week 6. Cumulative mortalities in oysters held at 18–30 ppt ranged from 34–41% after 4 weeks of exposure. Mortalities in oysters from the control and 14 ppt aquaria ranged from 1–4%. Only oysters from the control and 14 ppt aquaria failed to produce conchiolinous shell lesions indicative of JOD. Although salinity appears to be a factor in JOD, we have recently seen one instance where JOD-infected oysters survived months of exposure to low salinity water (<5 ppt) in Maryland and expressed the disease when placed in aquaria at 26 ppt.

Expanding on the premise that water filtration may exclude the JOD infective agent, field filtration experiments were designed and set up at two Long Island, NY sites. Oysters were exposed to unfiltered and double bagged, 50 and 25  $\mu\text{m}$  filtered JOD-infected water at the F. M. Flower & Sons Oyster Co. Bluepoints oysters initially raised in high salinity well water were exposed to infected unfiltered and 5  $\mu\text{m}$  filtered water at the Cornell University, Suffolk County Marine Environmental Learning Center (SCMELC). Suitable growth was not achieved in oysters at the SCMELC site to complete this part of the study. Assessed by mortalities and conchiolinous shell lesions, the JOD agent infected oysters in all three levels of filtration at the Flower site. However, mortality onset was delayed 1–2 weeks in 25  $\mu\text{m}$  filtered water. Susceptible oysters, spawned from Connecticut broodstock and held in 25  $\mu\text{m}$  filtered water, also suffered much lower cumulative mortalities (31%) than the same oysters in 50  $\mu\text{m}$  filtered (82%) or unfiltered water (45%). Oysters in filtered water experienced less mortality than smaller size-screened oysters from the same spawning batch reared commercially in grow-out trays.

A companion filtration experiment was set up in laboratory aquaria to attempt JOD transmission without the presence of JOD-infected oysters. The experimental source of infection was material retained in sequentially filtered 50, 25, and 5  $\mu\text{m}$  bag filters, plus what passed the 5  $\mu\text{m}$  filter. Mortalities were first observed during week 4 of the study in oysters exposed to material held by the 25 and 5  $\mu\text{m}$  filters, and passing the 5  $\mu\text{m}$  filter. Heaviest JOD-induced mortalities occurred in oysters exposed to the 5  $\mu\text{m}$  material (64%), followed by <5  $\mu\text{m}$  material (28%), 25  $\mu\text{m}$  material (16%), and 50  $\mu\text{m}$  material (3%). Control oysters suffered the lowest mortality (2%) with no indications of JOD. This, along with our previously presented histological data, continues to support the concept that the JOD disease agent may be a protistan parasite in the 5  $\mu\text{m}$  size range.

JOD-infected oysters from New York, Rhode Island, and the aquaria filtration transmission experiment were tested for *Vibrio*. As in our 1993 study, no specific *Vibrio* sp. was found to be associated with JOD. The inconsistency of *Vibrio* isolates, and

their lack of susceptibility to an antibiotic that drastically improved survival of JOD-infected oysters, further indicate the bacteria are secondary invaders and play an opportunistic role in this disease.

**COMMERCIAL RECIRCULATING SYSTEMS FOR FISH CULTURE IN THE NORTHEAST.** Scott Lindell and Josh Goldman, AquaFuture, Inc., P.O. Box 783, Turners Falls, MA 01376.

Some of the successes and failures of commercial recirculating fish culture in the Northeast are reviewed. Pitfalls of past commercial failures will be discussed with recommendations for future avoidance. The essential features of successful operations are presented including system design and management, feed and water quality considerations, and disease management.

**THE BIOLOGY AND ECONOMICS OF BAITFISH PRODUCTION IN POULTRY-PROCESSING WASTE-WATER TREATMENT LAGOONS.** William W. Lussier, Conrado M. Gempesaw II, and J. Richard Bacon, Department of Food and Resource Economics, University of Delaware, Newark, DE 19717.

Production of baitfish, primarily the golden shiner, *Notemigonus crysoleucas*, is one of the leading aquaculture industries in the United States. Production in Arkansas, where the majority of baitfish is produced, exceeded 14 million pounds and had a total value of approximately \$40 million dollars in 1993. The wholesale price for golden shiners in the Northeast and Mid-Atlantic region can range from \$18 to \$35.00 per kilogram, 3 to 6 times that of some species of food fish. During 1993–94, research was conducted at a Mid-Atlantic poultry processing plant using unfed golden shiners to determine the biological and economic viability of producing baitfish in the company's waste-water treatment system. The study found that the aquatic environment of waste-water treatment lagoons poses serious challenges to aquaculture production. Within the lagoon studied, ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ) reached 0.80 mg/L during 1993 while dissolved oxygen concentration ranged from near zero to supersaturation over the course of 24 hours. Although mortality exceeded 90%, fish that survived grew at a rate of 0.33 mm/day, a rate similar to the species grown under normal culture conditions. The financial performance of baitfish production was assessed using AQUASIM, a dynamic, stochastic, capital budgeting simulation model. The model found that baitfish production in waste-water treatment systems has a very high probability of economic success due to low costs of site development and integration of labor, overhead, and other costs into the company's established infrastructure. Future research should address the development of tolerant strains of golden shiners, fry production, and the impact of aquaculture on the quality of effluent discharged from the water treatment system.

**SOME FACTORS CONTROLLING NATURAL POPULATIONS OF SOFTSHELLS, *MYA ARENARIA*, WITH RECOMMENDATIONS FOR CULTURE.** Clyde L. MacKenzie, Jr., U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Laboratory, Building #74, Magruder Road, Highlands, NJ 07732.

Heavy setting of the softshell clam, *Mya arenaria*, in northeastern New Jersey occurred in 1993 and 1994. Successful sets seem to be related to weather conditions. The weather in both years was warm and dry, skies usually were clear, water temperatures increased steadily, and winds were gentle, with no northeast storms in spring or early summer.

The 1993 year class of softshells survived well through the cold winter of 1993–94 and hot summer of 1994, except where dense growths of sea lettuce, *Ulva lactuca*, covered the beds. In such areas, all the softshells died when concentrations of O<sub>2</sub> fell to nearly 0 ppm and water temperatures were 24–29°C in July.

In the Navesink and Shrewsbury Rivers, the 1994 year class was mostly eliminated by predation by *Fundulus majalis* and *Fundulus heteroclitus*. Blue crabs, *Callinectes sapidus*, also were likely predators. In Raritan Bay, where the 1994 density of juveniles was about 24,000/m<sup>2</sup> in an area at least 8 km long and 150 m wide, they were preyed upon by fish and blue crabs. Most survived the summer, but storms were responsible for the mortality of nearly all juveniles in the fall.

The naturally-set softshells could be cultured by removing sea lettuce from the beds, and by transplanting dense concentrations of seed to sheltered areas where they could be harvested after growth to market size. *Fundulus* sp. can consume softshells as large as 10 mm, and thus hatchery-reared softshells and cultured natural sets would need to be protected from the fish up to this length.

**BAY SCALLOP RESEARCH IN FLORIDA.** Dan C. Marelli, William S. Arnold, Catherine Bray, and Melissa Harrison, Department of Environmental Protection, Florida Marine Research Institute, 100 Eighth Avenue S.E., St. Petersburg, FL 33701–5095.

Southern bay scallop (*Argopecten irradians concentricus*) populations have been declining along peninsular Florida for decades. Although reasons for this decline are not completely known, probable causes include overharvest, recruitment failure, destruction of habitat, and deteriorating water quality.

We began monitoring a single scallop population in 1992 and expanded that effort to 6 populations along Florida's Gulf coast in 1994. We characterize the abundance and distribution of populations at St. Andrews Bay, St. Joseph Bay, Steinhatchee, Homosassa Bay, Tarpon Springs, and Pine Island Sound. In populations that are being actively harvested, we conduct this survey prior to and following the harvest season. Survey data allow us to determine whether a population is depauperate, declining, recovering, or stable and abundant. Our data have already led the Florida

Marine Fisheries Commission to propose significant changes in the bay scallop rule.

We are also monitoring the reproductive state and recruitment rates of the populations at Steinhatchee, Homosassa Bay, and Tarpon Springs to determine the relationship between spawner stock and recruitment—data that may allow us to predict the size of subsequent populations. Future research will examine the relationship between scallop density and spawning success and the efficacy of using cultured scallops to enhance the natural spawner stock in decimated populations. We also plan to examine the genetic composition of several Florida populations and the rates of gene flow between them.

**PREVALENCE OF *PERKINSUS* SP. IN CHESAPEAKE BAY SOFTSHELL CLAMS (*MYA ARENARIA*).** Shawn M. McLaughlin and C. Austin Farley, Oxford Field Station, Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA, 904 S. Morris Street, Oxford, MD 21654–9724, Roy F. Scott, Maryland Department of Natural Resources, Annapolis, MD 21401.

*Perkinsus marinus*, a protozoan parasite, causes disease in the eastern oyster, *Crassostrea virginica*, along the Atlantic coast and Gulf of Mexico. *Perkinsus* spp. have been reported in over 30 other species of mollusks including softshell clams (*Mya arenaria*) without causing significant mortalities. Examination of histological tissues from softshell clams collected during surveys in the Maryland portion of the Chesapeake Bay from 1965 to 1989 showed only rare occurrences of *Perkinsus* sp. in over 5,000 softshell clams collected from 20 sites. However, in December 1990 two softshell clams from a Swan Point sample were diagnosed with *Perkinsus* sp. based on rectal thioglycollate tests. *Perkinsus* sp. has since been identified in softshell clams from 7 additional Chesapeake Bay sites. Prevalences of *Perkinsus* sp. in Chesapeake Bay softshell clams peaked in 1991 and 1992, ranging from 3–53%, decreased in 1993 to a range of 3–17%, and decreased to zero in 1994. Respectively, salinities and temperatures at positive sites at the time of collection ranged from 7.5–12 ppt and 8–28°C in 1991, 5–16 ppt and 9–23°C in 1992, and 10–14 ppt and 14–25°C in 1993. The prevalence of *Perkinsus* sp. infections in softshell clams follows seasonal patterns observed in oysters, with prevalences peaking in the fall and declining in January through May.

In oysters, *P. marinus* cells are cytozoic in hemocytes and possibly epithelial cells, facilitating the distribution of the parasite throughout the tissues. In softshell clams, however, *Perkinsus* sp. appears typically to be isolated and encapsulated in a manner similar to the response reported in the Baltic clam, *Macoma balthica*. In early infections, *Perkinsus* sp. is concentrated in the gills of softshell clams. The parasite may also be found in digestive diverticula, kidneys, and gonads. Unencapsulated *Perkinsus* sp. may be observed in softshell clam tissues in advanced infections. Softshell clam mortalities due to *Perkinsus* sp. may occur when

clams are heavily infected and/or stressed by other diseases and environmental factors.

**MODIFYING OYSTER HATCHERY MANAGEMENT TO AVOID LOSSES DUE TO UNEXPLAINED MORTALITY OF JUVENILE OYSTERS: A 1994-UPDATE.** David Relyea, Frank M. Flower & Sons Inc., P.O. Box 1436, Bayville, NY 11709.

Starting in July of 1990, juvenile oysters in the growout raft system at Frank M. Flower and Sons in Bayville, N.Y. showed unusually high mortalities. Hatchery production declined by 50%. This pattern continued in 1991 and 1992. During this time much was learned about Juvenile Oyster Mortality (JOM) with the help of experienced marine scientists and our own observations. With this knowledge, hatchery procedures were modified in 1993. This resulted in substantially increased hatchery production as reported at the 1994 Milford Aquaculture Seminar. Building on this success, a similar strategy was used in 1994. This resulted in a record hatchery production year. Although the exact cause of JOM has not been identified, research continues. This presentation will illustrate how progress was made despite the continuing mystery of the causative agent.

**TRANSIENT-GEAR—A NOVEL APPROACH TO SHELLFISH AQUACULTURE.** Robert B. Rheault, Spatco, Ltd., 1121 Mooresfield Rd., Wakefield, RI 02879, Michael A. Rice, Department of Fisheries, Animal, Veterinary Science, University of Rhode Island, Kingston, RI 02881.

In New England one of the primary obstacles to the growth of aquaculture is the resistance to the leasing or privatization of public waters. For the past 3 years, Spatco, Ltd. has been testing a novel method of oyster aquaculture that we call "Transient-Gear Aquaculture." The method involves placing hatchery-reared shellfish in cages resembling lobster pots on the pond bottom. The cages are marked by buoys and hauled every few weeks for cleaning and tending. Since the gear is transient and periodically relocated, even the wild shellfish resource directly underneath the "oyster pots" eventually becomes accessible to the wild-harvest digger. This method of aquaculture is designed to avoid the need for exclusive use of leased bottom. If the economics of transient-gear aquaculture can be demonstrated, it is conceivable that many areas now closed to aquaculture because of public resistance to leasing will become open to productive, sustainable shellfish aquaculture.

The concept and methods are described and a model based on 4 years of data is presented. Based on an annual planting of 200,000 oyster seed, 2-to-3 years to market size, a 25% cumulative mortality, and a piece price of 40¢; the model predicts annual gross revenues of \$45,000 in the second year and \$61,000 each year thereafter, with sales of 3000 pieces per month. The model predicts that it would be necessary to deploy 129 to 225 of the twelve-bag (each bag is 24" square) pots to produce this crop.

Interactions of seston flux and shellfish growth will influence optimum stocking densities for each site, and will determine the amount of gear needed to produce this crop.

Compared with conventional approaches to shellfish aquaculture the method has significant advantages in terms of quality control, elimination of predation losses, and the ability to utilize areas of non-productive or even anoxic bottom. The economics of the approach suffer from high labor costs associated with maintaining the gear, however economies of scale and the premium price offered for top quality shellfish can compensate for those costs.

**SPATIAL AND TEMPORAL DISTRIBUTION OF BIVALVE LARVAE IN GREENWICH BAY, RHODE ISLAND, DURING THE SUMMER OF 1993.** Michael A. Rice and Joseph E. Goncalo, Department of Fisheries, Animal, and Veterinary Science, University of Rhode Island, Kingston, RI 02881.

Greenwich Bay, in Narragansett Bay, RI, is known to be an area that has supported recreational and commercial fisheries of the northern quahog, *Mercenaria mercenaria*. Sustained annual catches of approximately one million pounds of mostly smaller-sized "littlenecks" in the bay suggest annual recruitment, but few studies of early life history stages have been undertaken in this area since Landers showed maximum bivalve abundance occurred during the month of June in 1951 and 1952. Weekly water samples (100-L each) were taken on an incoming tide from 0.3 and 1.5 m depths at seven locations in Greenwich Bay: Greenwich Cove, Mary's Creek, Nausauket, Oakland Beach, Goddard Park, Sally Rock, and the mouth of Greenwich Bay. The Greenwich Cove and Mary's Creek sites were chosen because they are closed to shellfishing and have large adult quahog populations in excess of 90 animals/m<sup>2</sup>. Samples were taken using a 20 L/min, 12V-electric bilge pump and passing the water through a 60-µm mesh plankton net. Larvae were fixed in the field with 10% buffered formalin in filtered seawater and transferred to a 25% ethanol/seawater mixture for storage. Larvae in duplicate 1.0 mL subsamples of the preserved samples were identified and counted. Larvae from several invertebrate and vertebrate taxa were identified. Identifiable bivalve larvae were distinguished as to development stage: D-hinge veliger, umbonate veliger, and pediveliger. Maximum bivalve abundance occurred June 14 at all of the sites with a bay-wide average of 7,800 larvae/100L. A secondary peak of abundance occurred August 3 with 580 larvae/100L. The D-hinge veligers were much greater in number than umbonate veligers, which in turn were greater in number than pediveligers. This suggests predation loss or export of the developing larval stages. Larvae were not uniformly distributed throughout Greenwich Bay. Maximum bivalve larval abundances were found in the open bay sites rather than in coves and inlets. This is a surprising result because presumed "spawner stocks" reside in the coves. Further studies are needed to identify the most probable sources of larvae

leading to quahog recruitment in Greenwich Bay. This is publication 3044 of the Rhode Island Agricultural Experiment Station.

**EFFECTS OF FLOW AND STOCKING DENSITY ON GROWTH RATES AND SURVIVAL OF EASTERN OYSTERS WITH JOD.** **Gregg Rivara**, Cornell Cooperative Extension, Suffolk County, Southold, NY 11971, **Stanley Czyzyk**, Bluepoints Company Inc., P.O. Box 8, West Sayville, NY 11796.

Juvenile oyster disease (JOD) first affected the east coast (USA) oyster industry in 1988. Losses of close to 100% have occurred in seed less than 25 mm shell height. This experiment was designed to confirm earlier work that showed relatively high flow rates and low stocking densities in upweller nursery silos reduced mortalities related to this disease. A three tank, 27 silo upflow nursery system was constructed. Each tank served as a flow treatment: low (4 L/m per silo), medium (20 L/m) and high (40 L/m). Within each tank 3 target stocking densities were triplicated: low (one liter per silo), medium (six liters) and high (12 liters). Silo tanks were fed unfiltered creek water from a 3.4 m<sup>-3</sup> head tank filled by two 2 hp pool pumps. Silo units were initially stocked on 11 July 1994. Shell height at this time averaged 8 mm and stocking densities were well below targets. Each week, for a period of 9 weeks, the volume and mortality (dead/100 animals) of each silo, along with shell height measurements (live and dead) were recorded. Physical parameters measured included temperature, salinity, secchi depth and a check of flow rates. As the actual volume exceeded the target volume for each silo, oysters were randomly removed to bring the volume back down to the treatment level.

Substantial mortalities (>20%) were seen by week three in low flow silos; by week four mortalities in these silos averaged 60%. At this time high flow silos were at or under 33% dead, and had not increased substantially by the end of the trials. Weekly volumetric increases were higher in high and medium-flow silos as compared to low-flow silos. Flow affected growth and survival more than stocking density. It is not known to what degree nutritional stress caused by low flows and high densities affects the manifestation of JOD. It is suggested that silos can be highly stocked with relatively high flow rates (0.03 l<sup>-1</sup> cm<sup>-2</sup> silo screen area) in order to reduce mortalities of oysters exposed to JOD.

**HATCHERY PRODUCTION OF EASTERN OYSTER, CRASSOSTREA VIRGINICA, SEED: A BIOLOGICAL AND ECONOMIC INVESTIGATION OF LARVAL CULTURE TECHNIQUES INCLUDING PRELIMINARY OBSERVATIONS OF OPTIMAL STOCKING DENSITY AND SUBSTRATE SIZE IN A HATCHERY SETTING SYSTEM.** **Kimberly B. Simmons**, **Valerie L. Shaffer**, **Sayra G. Thacker** and **Mark W. Luckenbach**, The College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA 23062.

Optimization of culture and settling techniques offer the promise of improving economic viability of hatcheries for *Crassostrea virginica*. Separate experiments were conducted, under mass pro-

duction conditions, to determine the effects of various larval algal diets, setting system stocking density, and settlement substrate size on growth, survival, and strike occurrences. In all experiments, larvae from single cohorts were used.

The first study compared 2 larval diets: algae grown with low cost and labor input, and algal growth with high cost and high labor input. The low-input larvae were cultured on low nutrient Tahitian *Isochrysis galbana* (T-iso) exclusively, while the high-input larvae were cultured on high nutrient T-iso, *Chaetocerus calcitrans*, and *Tetraselmis maculata*. Feed rations for the low-input larvae ranged from 10,000 cells/mL to 20,000 cells/mL per day; while the high-input larvae feed rations ranged from 30,000 cells/mL to 60,000 cells/mL per day. Cost of seed production was compared for the 2 larval culture methods (with post-set conditions held constant) to determine optimal return on investment. Increased initial expenses associated with the high-input larvae were offset by improved growth and survival.

Optimal stocking density and substrate size were investigated using single cohort larvae added to downweller units coated with paraffin wax. Seven substrate treatments were tested with size operationally defined by retention of crushed oyster shell on 0.3, 0.5, 1.4, 2.0 and 4.75 mm sieve series, as well as a mixed range (1.0–2.0 mm) treatment. Two hundred and ten thousand pediveligers were placed into each of 3 replicate downweller units containing the appropriate substrate and allowed to settle. Various stocking densities ranging from 12,400–150,000 pediveligers per downweller unit were tested using a single substrate size (0.5 mm). Maximum survival and minimal multiple strikes were observed on the 0.5 mm crushed shell. These findings suggest directions for optimizing the diet and clutch size. Further investigations are required to elucidate optimization of stocking densities.

**CILIATED PROTISTS ASSOCIATED WITH JUVENILE OYSTER DISEASE.** **Eugene B. Small**, Department of Zoology, University of Maryland, College Park, MD 20742–4415.

Protists were successfully stained and identified to genus (for the ciliates encountered) for collections made from two sites—The Frank M. Flower Oyster Hatchery Facility seed rack containers; the filtered particulates in which the particles were passed through a 47 micrometer filter but retained by a 5 micrometer filter; and the particulate residue in the experimental tanks at the Oxford Laboratory in which JOD killed more than 80% of the oysters by 6 weeks and about 75% of the juvenile oysters by 6 weeks for salinities of 26 ppt and 20 ppt, respectively.

In these experimental tanks only a small, 9–11 micrometers diameter, suctorian ciliate, *Endosphaera* sp., was recovered and successfully stained via the Protargol staining technique. Also found in these samples was a small unknown (new to science) stichotrich ciliate containing similar suctorians within the cytoplasm of the stichotrich ciliate. The size of the internally encountered suctorians was in the order of 5–8 micrometers, about the same size as the eukaryotic endoparasites earlier encountered in

the separate studies of Farley and Lewis as seen in histological feulgen stained tissues in which suspect macro- and micronuclei were seen in addition to healthy host nuclei in sectioned mantle epithelium. A similar small (about 11 micrometers) suctorian (identified as *Endosphaera* sp.) has most recently been found in the same particulate fraction of filtered particulates from which Farley and Lewis have successfully infected juvenile oysters this past summer of 1994.

From the mantle cavity fluids of JOD infected oysters, new species of *Mesanophrys* and *Paranophrys* have been discovered. Both of these genera contain ciliate species known to be parasites of other invertebrate hosts (eg. *Mesanophrys pugettensis* in Dungeness Crabs on the northwest Pacific Coast, and *Mesano-phrys* sp. in Chesapeake Bay blue crabs and the gonads of the hydrozoan *Tubularia* sp. from Woods Hole, MA). However, these apparent histophagous ciliated protists of various invertebrates are too large to be directly related to the intracellular mantle tissue parasites first observed by Farley and Lewis. The known intracellular suctorian, *Endosphaera* sp., is, however, the prime suspect.

**POSSIBLE CYTOTOXIC EFFECTS OF THE DINOFLAGELLATE, *GYRODINIUM AUREOLUM*, ON JUVENILE BIVALVE MOLLUSCS.** Roxanna Smolowitz, Laboratory for Marine Animal Health, Marine Biological Laboratory, Woods Hole, MA 02543, Sandra E. Shumway, Natural Science Division, Southampton College of Long Island University, Southampton, NY 11968.

Juveniles of 8 commercially important species of bivalve molluscs (surfclam, *Spisula solidissima*; bay scallop, *Argopecten irradians*; eastern oyster, *Crassostrea virginica*; blue mussel, *Mytilus edulis*; softshell clam, *Mya arenaria*; European oyster, *Ostrea edulis*; northern quahog, *Mercenaria*; sea scallop, *Placopecten magellanicus*) were exposed in the laboratory to the commonly occurring dinoflagellate, *Gyrodinium aureolum*. Histological analyses of gut tissues indicated that the impact of this dinoflagellate on the shellfish was species-specific. High rates of mortality were noted in the bay scallop (*A. irradians*), but not in other molluscan species exposed to *Gyrodinium*. There were no pathological differences between control animals and animals fed *Gyrodinium* in *S. solidissima*, *M. arenaria*, or *M. mercenaria*. There appeared to be a difference in digestive gland parameters between control and experimental mussels (*M. edulis*) after day 3; however, this difference did not exist after 7 days exposure and may represent an adjustment by the mussels fed *Gyrodinium* to this new food type. There was also a suggestion of reduction of height of absorptive cell epithelium and lumen size and some mortality in *P. magellanicus* after exposure to *Gyrodinium*; however, these results were inconclusive.

The 2 most severely affected molluscs were *C. virginica* and *A. irradians*. While *C. virginica* did not exhibit any differences in digestive gland parameters between control and experimental animals, several animals did show significant mantle and gill lesions.

It is not clear whether these lesions are a result of exposure to *Gyrodinium* or are unrelated and coincidentally occurred in the population. Bay scallops (*A. irradians*) exhibited decreased height of absorptive cells and increased lumen diameter after exposure to *Gyrodinium* suggesting, at least, poor food quality of *Gyrodinium*. Evidence of toxic effects was not identified in the digestive gland. Several bay scallops also showed variable amounts of inflammation in the kidney associated with protozoal infestations and variable amounts of predominately rod-shaped bacteria within the urinary space. It is not clear if the kidney lesions are a result of debilitation of the scallops, co-existent, or causative for the digestive changes caused by dinoflagellate feeding (possible renal toxin).

Aquaculturists, especially of scallop species, should monitor for the presence of *G. aureolum*. Given its large size (25–30  $\mu\text{m}$ ), *G. aureolum* could easily be filtered from incoming water to hatcheries, thus avoiding mass mortalities of spat and juvenile scallops.

**THE DETERMINATION OF OPTIMAL SETTLEMENT AND RECRUITMENT OF BAY SCALLOPS, *ARGOPECTEN IRRADIANS*, TO ARTIFICIAL SPAT COLLECTORS IN THE WESTPORT RIVER ESTUARY, MASSACHUSETTS.** Karin A. Tammi and Michael Rice, Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI 02881, Scott Soares, SRPEDD, 88 Broadway, Taunton, MA 02780, Wayne Turner, Water Works Group, P.O. Box 197, Westport Point, MA 02791, Margaret Brumsted, Dartmouth High School, Slocum Road, Dartmouth, MA 02747.

In response to poor recruitment of the bay scallop, *Argopecten irradians*, in the Westport River estuary, Massachusetts, the Bay Scallop Restoration Project was initiated in 1993. The main objective of the project is to enhance scallop stocks by using spawner rafts and artificial spat collectors. Artificial spat collectors (2 to 4 mm plastic mesh bags containing monofilament) were suspended on 25 to 35 ft. floating longlines at study sites within the estuary. Along with biotic and physical factors, high variability between sites greatly influenced the assessment of productivity, determination of settlement times and evaluation of recruitment estimates for all study areas.

Research conducted in 1994 focused on improving the methods from the 1993 experimental study. During the summer 1994, spatlines containing 20 spat collectors were sequentially deployed at 8 study sites. A single spatline was deployed weekly to each area for a period of 10 weeks to determine peak settlement and recruitment times and evaluate fouling and mud crab predation. A total of 80 spatlines and 1600 spat collectors were placed within the Westport estuary (each study site containing a total of 10 lines and 200 spat collectors). The September to October 1994 harvest yielded 1088 scallops. The most productive study sites were Corey's Island (492 scallops), Canoe Rock (182 scallops), Jug Rock (106 scallops) and Southard Shore (104 scallops). The best recruitment for any of the study sites was observed at Corey's Island which yielded a total of

492 scallops. In general, the greatest recruitment to all 8 study sites occurred on spatlines deployed from weeks 6 to 9 of the experiment. Spatlines deployed in week 7 at Corey's Island displayed the greatest individual spatline recruitment, averaging 6.8 scallops per bag. Both Speaking Rock and Hick's Cove had the poorest scallop recruitment, but displayed the greatest volume of mud crabs, *Panopeus* spp. (16,520 mL and 14,375 mL, respectively).

This study indicates that *A. irradians* will settle onto artificial substrate and suggests that poor recruitment at some sites may be attributed to fouling, crab predation, and possibly estuarine circulation patterns. This information will enable researchers to improve spat collection efforts for the 1995 season by optimizing the deployment time of collectors in the most productive study sites. Consequently, spat harvested from the collectors could be utilized for grow-out purposes such as spawner rafts, or may be re-seeded into the estuary to enhance natural stocks.

**“BAGS TO DRAGS,” THE BAY SCALLOP RESTORATION PROJECT IN THE WESTPORT RIVER, A TWO-YEAR UPDATE.** Wayne H. Turner, The Water Works Group\*, P.O. Box 197, Westport Point, MA 02791.

In an effort to return the universally appealing and economically valuable bay scallop resource to the Westport River, The Water Works Group founded the Bay Scallop Restoration Project (BSRP). Spawned in January of 1993, the BSRP has devised and set into action an innovative bay scallop propagation program employing uniquely simple equipment and a large cast of community players.

“Spawning sanctuaries,” used to concentrate broodstocks, and “spat bags,” designed to collect a sample of the juvenile offspring as a method of replenishing the broodstock and indexing the wild set, have allowed the BSRP, in its first year, to amplify the brood stock by tenfold. The wild set has been substantial and in 1994 Westport enjoyed its first measurable bay scallop harvest in nine years. Furthermore, spat bags deployed during the summer of 1994 are forecasting even greater wild recruitment for the 1995 bay scallop season.

Speculated to be the origin of Westport's new found bay scallop production, spawning sanctuaries encourage “mass spawning” events. As such, a graduate student from the University of Rhode Island (URI) has begun research to measure the impacts of spawning sanctuaries on recruitment to the shellfishery in Westport. In tandem, graduate students from URI have also been working on a 2-year project aimed at translating bay scallop settlement on spat bags to wild recruitment (data presently under analysis).

Faculty, graduate, and undergraduate students from a host of universities have been brought aboard to address various aspects of bay scallop propagation, pollution remediation, and watershed management. In support of these initiatives, local town boards and agencies, including the Shellfish Department and the Board of Health, the Massachusetts Division of Marine Fisheries, a substantial number of local business and volunteers from the Westport

Fishermen's Association, the Westport River Watershed Alliance, and the general public as well as more than 1,500 students and teachers from schools of five surrounding communities have provided necessary materials and equipment while investing more than 30,000 volunteer hours in the effort.

\*The Water Works Group is a 501 (c) (3) nonprofit organization working to restore, maintain, and improve the economic, recreational, and aesthetic values of watersheds for the benefit of the public: present and future.

**POPULATION RESPONSE OF THE SOFTSHELL CLAM, *MYA ARENARIA*, TO INJECTION OF DISEASED HEMOCYTES.** James R. Weinberg, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543, Dale Leavitt and Judith McDowell Capuzzo, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Studies by Leavitt et al. (1990) and Brousseau and Baglivo (1991) documented the prevalence and lethal nature of hemato-poietic neoplasia in *Mya arenaria* populations from Buzzards Bay, MA and Long Island Sound. The current study was undertaken to explore 3 issues: 1) transmission of disease via injection of diseased hemocytes, for use as a tool to conduct controlled experiments, 2) differential impact of the disease on clams from different size classes, and 3) comparison of results from field and laboratory experiments.

A large-scale field experiment, involving about 2000 individuals, was carried out in New Bedford Harbor, MA from January 1990 to July 1992 (555 days) using 3 size classes of clams: 20–30, 30–40 and 40–50 mm. These clams were collected originally from Little Buttermilk Bay where prevalence of disease is relatively low. Clams were measured and assigned randomly to receive either an injection of filtered sea water (controls) or an injection of hemocytes from diseased individuals (treatment). After injection, clams were labelled and returned to the field.

In the field experiment, prevalence of disease in the large size class was greater in the treatment group than in the controls during the summer season; this suggests that the injection induced the disease as intended. Among clams in the control group, the probability of survival decreased with clam size. Relative to controls, the treatment significantly reduced the survival rate of large and medium-sized clams, but not that of small-sized clams. This suggests that clam size (and age) should be included as a factor in these studies. No difference was found between the treatment and control groups with respect to weight of somatic tissue, gonads, or shell growth. This is attributed to the fact that a substantial number of individuals died from the “diseased hemocyte” treatment, the same individuals that probably would have had lower weights and shell growth if they had survived to be measured.

Very different results were obtained when the experiment was conducted in the laboratory. No clams died during the 4-month

long laboratory experiment, and the "diseased hemocyte" injection treatment had no detectable effect on disease prevalence or on clam tissue weight, shell growth, or survival rate.

#### CONSUMER PREFERENCES FOR FRESH SHELLFISH: IMPLICATIONS FOR SHELLFISH MARKETING.

**Cathy R. Wessells**, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881, **Conrado M. Gempesaw II** and **J. Richard Bacon**, Department of Food and Resource Economics, University of Delaware, Newark, DE 19717, **Alberto Manalo**, Department of Resource Economics and Development, University of New Hampshire, Durham, NH 03824.

Aquaculture producers typically face challenges of foreign and domestic competition in the U.S. market for their products. Given current and projected future supplies, increases in revenues to producers of aquaculture seafood products must be generated within the marketplace. One way to affect prices received is to increase demand for the product. However, in order to increase demand, it is first necessary to obtain a better understanding of the factors which drive demand.

Today's seafood consumers have a wide array of products to choose from, made up of many species supplied by domestic production and imports, those harvested from wild fisheries and those produced from aquaculture operations. Consumer choices regarding seafood purchase decisions are affected by many factors, including economic factors such as price and income, socio-demographic factors such as ethnicity, household composition and location, and preferences and beliefs regarding seafood products.

In this presentation, data generated from a mail survey of 1,529 households in the Northeastern and Mid-Atlantic regions of the U.S. is used to analyze factors which influence consumers' choices regarding purchase decisions of mussels, clams and oysters. Analysis suggests that consumption of shellfish products is significantly influenced by several factors, including perceived quality and safety of the product. Implications of these results are discussed, with an emphasis on how the shellfish aquaculture industry may use these results in their marketing strategies.

#### RAPID GROWTH OF POST-SET OYSTERS AND SCALLOPS FED HIGH-LIPID *TETRASELMIS* CULTURES.

**Gary H. Wikfors**, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Avenue, Milford, CT 06460, **Glenn W. Patterson**, Department of Botany, University of Maryland, College Park, MD 20742, **Ralph A. Lewin**, University of California, Scripps Institution of Oceanography, La Jolla, CA 92093.

Our previous studies have demonstrated a positive correlation of growth of the eastern oyster, *Crassostrea virginica*, with contents of the essential fatty acids (20:5n3 and 22:6n3) and certain delta-5 sterols in diets of cultured phytoplankton. This correlation led us to screen marine microalgal strains for these biochemical

compounds in an effort to identify promising nursery diets for bivalve shellfish. Marine algae isolated selectively for high lipid content were analyzed for fatty-acid and sterol contents, and several were found to contain appreciably more of the specific lipid compounds of interest; the best of these were from the prasinophyte genus, *Tetraselmis*.

We conducted feeding studies comparing growth of both oysters and bay scallops, *Argopecten irradians*, on the high-lipid *Tetraselmis* strains and other algal strains used widely in molluscan hatcheries. Five *Tetraselmis* strains were found to support oyster growth that was significantly and appreciably more rapid than other algal diets when fed at an equivalent ration. Three of the strains we tested with post-set scallops were superior diets for this mollusk as well. Sizes of the high-lipid *Tetraselmis* strains range from 9–15  $\mu\text{m}$ ; therefore they are not suitable for first-feeding larvae, but some may be useful as diets for older larvae. Division rates, temperature tolerance, and effects of growth conditions on nutritional value remain to be investigated. Nevertheless, we believe that these strains offer great potential as diets for hatchery and nursery culture of bivalve mollusks.

#### THE ROLE OF EPHEDRINE IN LARVAL METAMORPHOSIS OF THE BLUE MUSSEL, *MYTILUS EDULIS*.

**Qin-Zhao Xue**, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China.

Ephedrine is a neuro-transmitter which can excite the alpha-receptor in mammals. The potential for ephedrine to induce metamorphosis rapidly and efficiently, and thereby reduce larval settlement mortality in the blue mussel, *Mytilus edulis*, was investigated. Larvae used for the induction tests were reared in filtered seawater with antibiotics (penicillin, streptomycin) and EDTA, and were fed microalgae such as *Phaeodactylum tricornutum* and *Isochrysis galbana*. Veliger larvae with eye spots, which can reach the size for metamorphosis of 280  $\mu$  27 days after fertilization, were selected for the experiment.

Results indicated that ephedrine had a strong positive effect in inducing larval metamorphosis of the mussel. The percentage of metamorphosing larvae treated by ephedrine ( $10^{-5}\text{M}$ , 36 hr) was as high as 62.5% greater than the control. The most suitable concentration and treatment time of ephedrine should be less than  $10^{-4}\text{M}$  and 36 hr, respectively. In the control group, without any inducer, larval metamorphosis was delayed for up to 20 days (47 days post fertilization), with obviously retarded growth (ca.330  $\mu$ ), when compared with the ephedrine-treated group (>500  $\mu$ ).

Larval-settlement response, such as plantigrade activities to find a suitable substrate, was observed after the ephedrine was added to the seawater. To eliminate the influence of gravity in the induction of settlement, a trial of upwards induction of larval settlement and metamorphosis was carried out using an agar-inducer (1.5%) complex. The agar was melted, mixed with inducer, and suspended at the surface of the seawater. Although the results of upward settlement induction were not consistent, data

showed that ephedrine was very effective in inducing larval settlement and metamorphosis of the blue mussel, *Mytilus edulis*.

**PREVALENCE OF *BONAMIA OSTREAE* IN EUROPEAN OYSTERS IN THE DAMARISCOTTA RIVER, MAINE.**

**Adriana Zabaleta** and **Bruce J. Barber**, Department of Animal Veterinary and Aquatic Sciences University of Maine, Orono, ME.

*Bonamia ostreae*, an Haplosporidian parasite responsible for *Bonamia* disease, was observed in the Damariscotta River, ME, in 1991. Since June 1994 three locations along the Damariscotta River (Little Point, Mears Cove and Witch Island) have been studied in order to determine the prevalence and intensity of in-

fection of *B. ostreae* along this river, throughout the year. Results from the first 2 sample collections are given.

Samples of 25 oysters were collected on 10 June and 25 August 1994. All animals were fixed in Helly's fixative. Paraffin sections (5 mm) including digestive gland, gonad and gill tissues were stained with hematoxylin and eosin and observed under a microscope.

The infection prevalence, both in June and in August, was low at the 3 sites (8–12%). The average intensity of infection, according to the classification of Boulo et al. (1989) was very low (fewer than 10 parasites observed during 10 min examination) in all locations for both sample collections. Comparison of these results with those from previous years at the upriver location showed a decrease both in the prevalence and in the intensity of *B. ostreae* infection since 1991.



**ABSTRACTS OF TECHNICAL PAPERS PRESENTED AT AQUACULTURE '95\***

*Triennial meeting of:*

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**\*Editor's Note:** Since this meeting was topic-oriented rather than society-oriented, papers presented here represent those whose author(s) signified membership in the National Shellfisheries Association, or were invited for inclusion by the Editor.



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**RECENT ADVANCES IN BIVALVE GENETICS.** **Standish K. Allen, Jr.**, Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, Port Norris, NJ 08349.

This paper discusses some of the recent advances in shellfish genetics as they pertain to practical application in the field. For example, recent findings on genotype-environment interaction in shellfish have complicated strategies for selective breeding of traits like faster growth. Other data on the genetics of host-parasite interactions in eastern oyster and the pathogen, *Perkinsus marinus*, have positive implications for selective breeding for Dermo resistance, an especially important goal for the eastern US. New evidence on the field performance of triploid shellfish has shown that their growth advantage over diploids is probably environment dependent, shedding a more sober light on the commercial potential of triploids. The recent development of tetraploid shellfish offers the possibility of making a different kind of triploid if tetraploids produces diploid gametes as expected. Data from breeding trials of tetraploids thus far indicates that this is so. Advances in the molecular genetics of shellfish have produced diagnostic techniques for discriminating species (such as between *Crassostrea* spp.). In particular, the ability to discriminate between *C. gigas* Miyagi and *C. sikamea* (Kumamoto) using gene probes has broad practical potential on the west coast of the US where these two have been inadvertently (and purposefully) hybridized. Another advance in molecular markers is the development of microsatellites that have potential for marker assisted selection or for gene mapping. Especially useful would be locating genes for disease resistance, for example, to *Bonamia* or *Perkinsus*. Locating such genes might enable genetic engineering in shellfish. Steps in that direction were realized when the first successful insertion of foreign genes in *Mulinia lateralis* was accomplished only this year.

**GENOTYPE-SPECIFIC GROWTH OF HARD CLAMS (GENUS *MERCENARIA*) IN A HYBRID ZONE: VARIATION AMONG HABITATS.** **W. S. Arnold, T. M. Bert and D. C. Marelli**, Florida Marine Research Institute, 100 Eighth Avenue S.E., St. Petersburg, FL 33701, **H. Cruz-Lopez**, Florida Game and Fresh Water Fish Commission, 801 Northwest 40th Street, Boca Raton, FL 33431.

Shell growth rate is an important component of fitness in bivalve molluscs. We compared rates of shell growth among the hard clams *Mercenaria mercenaria*, *M. campechiensis*, and their hybrids sampled from a variety of habitats in the Indian River lagoon, Florida, a zone of species overlap and natural hybridization. We verified the annual nature of shell growth increments in each of the 3 genotype classes in the lagoon; we then quantitatively compared annual growth rates within and between genotype

classes within and between habitats using the  $w$  parameter computed from the Von Bertalanffy growth equation. Our results indicate that the classical paradigm describing hard clam growth, in which growth rate is most rapid in *Mercenaria campechiensis*, intermediate in hybrids, and slowest in *M. mercenaria*, is not supported in the Indian River lagoon. Instead, *M. campechiensis* is at a growth disadvantage in most lagoonal habitats, possibly due to salinity stress, but maintains a growth advantage in deep-water habitats in the northern section of our study area. Similarly, hybrids maintain a growth advantage over *M. mercenaria* in shallow-water habitats in the central and southern sections of our study area. In all other habitats, either growth rate among genotype classes is equal, or *M. mercenaria* maintains a growth advantage. This complex relationship between genotype and habitat-specific growth provides a mechanism for environmentally mediated (exogenous) selection to act on hard clams in the Indian River.

**PRELIMINARY INVESTIGATIONS OF THE DISTRIBUTION, BIOLOGY AND POSSIBLE EFFECTS OF THE TURBELLARIAN *URASTOMA CYPRINAE* ON THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*.** **E. Bataller\***, **M. Clement** and **A. D. Boghen**, Université de Moncton, Department of Biology, Moncton, New Brunswick, Canada, E1A 3E9.

*Urastoma cyprinae* is a Turbellarian that has been reported on the gills of oysters in eastern Canada in numbers as high as 2000 worms/oyster. Aside from their aesthetic impact, previous studies suggest that the worms may also contribute to a general deterioration and an overall pathology of their host. This has already been confirmed to be the case for other molluscan species such as mussels.

The presentation provides an overview of a series of studies involving *U. cyprinae* and makes reference to the following: a) a general parasitological survey conducted in Canada along New Brunswick's east coast between May and October 1994 suggesting that the incidence and the infestation levels of *U. cyprinae* are site specific b) preliminary investigations indicating that heavily parasitized oysters display reduced feeding efficiency as determined by clearing rates of known levels of phytoplankton, c) LT50 trials conducted on isolated samples of *U. cyprinae* maintained at different salinity concentrations ranging from 10 ppt to 40 ppt and showing that the worms survive best at salinities of about 20 ppt and d) a comparative study of the incidence of *U. cyprinae* in oysters reared by different methods, demonstrating that only bottom-cultured animals become infected with the parasite. The implications of the findings are presented and proposals for future work discussed.

**IMPROVING SURVIVAL OF PLANTED JUVENILES OF THE GEODUCK CLAM (*PANOPEA ABRUPTA*) USING PREDATOR EXCLUSION DEVICES.** J. Harold Beattie\*, Brady Blake, and Dwight Herren, Washington Department of Fish and Wildlife, Point Whitney Laboratory, 1000 Point Whitney Road, Brinnon, WA 98320.

Washington Department of Fish and Wildlife (Formerly Washington Department of Fisheries) personnel have been culturing the geoduck clam since the 1970s. Staff at the Point Whitney Laboratory developed techniques for larval and juvenile culture. During the 1980s the scale of culture expanded to the production of millions of 10 mm seed geoduck per year. The seed were planted into subtidal areas by broadcasting them from the stern of a slowly moving boat. Upon reaching the substrate most of the juvenile geoducks dug in successfully without being preyed upon. Two years after these broadcast plants, dive surveys for young clams revealed few had survived. In almost every case the survival estimate was less than 1 percent. In searching for a cause for this poor survival, Point Whitney biologists performed predator experiments. The most striking results of these experiments revealed that during a 48 hour exposure period, crabs (*Cancer productus* and *Cancer gracilis*) would consume 30 to 40% of all sizes of geoduck seed tested. In testing various methods of protecting the seed from predators, hatchery personnel found an effective deterrent to predation: vertically installed PVC pipe. In 1991 on 4 State Park beaches, one foot long sections of 4-inch and 6-inch diameter PVC pipe provided protection for the geoduck seed. Average survival from these intertidal plants varied from 20 to 70% ten months after planting. Another type of protective device tested in subtidal areas was paper composite tree planting cones. These cones also proved to be effective in protecting the geoduck juveniles from predation. Recovery of geoducks planted in cones averaged 20% while recovery of geoducks planted without protection was 0%.

**THE SPERMATHECA OF IMMATURE, PRE-MATURE, AND MATURE SNOW CRABS, *CHIONOECETES OPILIO*.** Peter G. Beninger\*, Département de Biologie, Université de Moncton, Moncton, N.B. E1A 3E9, Carole Lanteigne, Centre marin de Shippagan, Shippagan N.B. EOB 2B0, Canada.

In order to better understand the reproductive biology of the commercially-fished species *Chionoecetes opilio*, the spermatheca of immature female snow crabs were examined using histology, histochemistry, and electron microscopy. The data indicated that female snow crabs could be divided into 3 categories: immature (approx. 20-50 mm carapace width, CW), pre-mature (approx. 40-60 mm CW) and mature (>60 mm CW). Immature crabs were still temporally remote from the terminal molt, had white ovaries,

and the spermatheca presented a thin tissular separation between the dorsal and ventral regions. No stratified glandular epithelium was present; only a columnar epithelium lined the lumen. Premature females were temporally close to terminal molt, had orange ovaries, and retained the tissular separation between the dorsal and ventral regions of the spermatheca. In addition, they presented a stratified glandular epithelium which developed beneath the columnar epithelium lining the lumen. The latter sloughed off into the lumen as the former developed. Mature females had undergone the terminal molt, had extruded eggs, and showed no trace of either the tissular separation between dorsal and ventral regions of the spermatheca, or the columnar epithelium which were present in the preceding 2 categories. These results extend our knowledge of the anatomical and physiological development of the reproductive systems in this species, and allow a more precise definition of sexual maturity.

**THE OSPHRADIUM IN *PLACOPECTEN MAGELLANICUS* AND *PECTEN MAXIMUS* (BIVALVIA, PECTINIDAE): HISTOLOGY, ULTRASTRUCTURE, AND IMPLICATIONS FOR SPAWNING SYNCHRONISATION.** Peter G. Beninger\*, Département de Biologie, Université de Moncton, Moncton, N.B. Canada E1A 3E9, Anne Donval and Marcel Le Pennec, Laboratoire de Biologie Marine, Université de Bretagne Occidentale, 29287 Brest Cédex, France.

Anatomical, histological, and electron microscopical techniques were used to elucidate the structure and ultrastructure of the osphradium in *Pecten maximus* and *Placopecten magellanicus*. The osphradium consists of 2 distinct regions in the gill axis: the osphradial ridge, and the dorsal region. The dorsal region contained free nerve fibers and ciliary tufts, separated by undifferentiated epithelial cells. The osphradial ridge was largely devoid of cilia other than those of the few free nerve fibers and very sparse ciliary tufts. Most of the cells of the osphradial ridge were highly secretory, the principal products being large pigment granules directly secreted by the Golgi bodies, and numerous small, electron-dense vesicles. These vesicles aligned along extensive microtubule arrays in the basal region, indicative of axonal transport. The data of the present study support and extend Haszprunar's hypothesis of the role of the osphradium in the reception of chemical spawning cues and in the synchronization of gamete emission. Together with independent data on nerve pathways, osphradial sensory modalities, and monoamine localisation, an anatomical pathway and neurophysiological mediator are postulated.

**EXPERIMENTAL CULTURE OF PEARL OYSTERS (*PINCTADA IMBRICATA* AND *PTERIA COLYMBUS*) (BIVALVIA: PTERIIDAE) ON THE CARIBBEAN COAST OF COLOMBIA.** Francisco T. Borrero, INVEMAR, Instituto de Investigaciones Marinas de Punta de Betin, A.A. 1016, Santa Marta, Colombia.

Two species of pearl oysters are being cultured experimentally in the Santa Marta region of Colombia. Studies of the reproductive biology of adult oysters, and of seasonal variations in larval abundance and spat settlement, seek to arrive at an appropriate schedule for culture activities. These species are protandric hermaphrodites, since after the minimum size for sexual activity, most individuals are male or functional hermaphrodites, and only the largest individuals contain exclusively female gametes. Natural populations have been found to differ in their sexual structure, and this is important for broodstock procurement and management. Two yearly spawning events have been documented for *P. colymbus*, and this is probably the case for both species. Settlement of *P. imbricata* spat on onionbag collectors is abundant in April-June and September-December, although small numbers of seed can be collected all year. *P. colymbus* settlement has been low on all types of collectors tested, but abundant on natural substrata from April through June. Growth of both species is higher in suspended culture than in the natural habitats, and high compared to rates reported for other cultured pearl oyster species. Experiments on controlled conditioning and spawning have been initiated to make seed available at all times and to areas where natural settlement is low. Cultured half-pearl production has been initiated, and monitoring of growth of grafted oysters, and of pearl quality is being compared in three different suspended culture systems. Pearl oyster cultivation promises to become an important source of employment and income for coastal villagers in Colombia, since markets for pearls, shells and meat appear quite favorable.

**THE ENIGMATIC SCALLOP PROTOZOAN PATHOGEN (SPX) OF CULTURED JAPANESE SCALLOPS, *PATINOPECTEN YESSOENSIS*, IN BRITISH COLUMBIA, CANADA.** Susan M. Bower\*, Gary R. Meyer, and Janice Blackburn, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada, V9R 5K6.

An exclusive protozoan of unknown taxonomic affinities (called SPX) sporadically occurs in Japanese scallops during grow-out in British Columbia. Infection results in mortalities that approach 100% for juveniles less than 5 cm in shell height. The disease is evident with creamy-white pustules in all organs, but most frequently in the connective tissue of the gonad, digestive gland, and mantle. Although SPX can infect scallops of all sizes, the biflagellated stage (about 3 mm in length) has only been observed in the edematous tissues spaces of about 15% of the infected small scallops (<3 cm shell height). At the anterior end of

the ellipsoid flagellated stage, an electron dense, rod-like structure occurs within the cytoplasm adjacent to the nucleus. This structure was reminiscent of rectilinear micronemes (apical complex) described in zoospores of *Perkinsus* (= *Dermocystidium*) *marinus* a pathogen of *Crassostrea virginica* on the east coast of the United States. However, there are three significant characteristics of SPX that differ from that of all known species of *Perkinsus*: (1) the thioglycollate culture test, which is diagnostic for *Perkinsus* spp. was negative for SPX, (2) the flagellated stage of *Perkinsus* spp. do not occur within the tissues of the molluscan host, and (3) to date all *Perkinsus* spp. have been reported from molluscs in the tropics or in warm temperate waters (SPX infections have developed in scallops in water at about 9°C). Transmission by injection of homogenized pustules, and by cohabitation with infected scallops occurred at relatively low levels (about 50% infected) with a prolonged prepatent period (about 40 days), only when flagellated stages were present in the source material. Although evidence suggests that SPX is enzootic to British Columbia, the natural host of SPX is not known. However, absence of SPX at some grow-out localities suggests that the distribution of SPX is restricted.

**COMPARATIVE PERFORMANCE OF CULTURED NATIVE AND SELECTIVELY BRED EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*, IN FLOATING RAFTS.** Bonnie L. Brown\*, Virginia Commonwealth University, Ecological Genetics Lab, 816 Park Avenue, Richmond, Virginia 23284-2012, Arthur J. Butt, Virginia Dept. of Environmental Quality, P.O. Box 10009, Richmond, VA 23240, Kennedy T. Paynter, University of Maryland College Park, Dept. of Zoology, College Park, MD 20742.

Performance of 2 eastern oyster strains, *Crassostrea virginica*, was compared in North Carolina, Virginia and Maryland during 1992-1994. Strains were selected to represent the types of oyster cultured by the maturing oyster mariculture industry along the US east coast: a wild strain from North Carolina and a selectively bred strain derived from upper Chesapeake Bay. Growth, response to infection with *Perkinsus marinus*, and mortality were each significant factors in performance and results differed markedly for the 2 strains. Oysters at all sites exhibited chronic Dermo infection. Mortality of the selectively bred strain cultured in high salinity was near 100% following the first summer Dermo challenge. Concurrent disease-related mortality of the wild strain held at high salinities was <35%. At low salinity, neither strain exhibited disease-related mortality. Average growth rate at high salinity was 5.3 mm/month for the native strain vs. 3.9 mm/month for the selectively bred strain. Average growth rate at low salinity was 4.0 mm/month for the native strain vs. 4.2 mm/month for the selectively bred strain. Native oysters required approximately 12 months to reach harvest size of 76 mm in high salinity. Selectively bred oysters reached harvest size first at low salinity, but required

22 months. Inverse growth trends suggest that performance of oysters is related to genetic makeup, while mortality trends suggest a relationship to other factors such as acclimatization and pathogenicity of *P. marinus*.

**SPATIAL DISTRIBUTION AND INTENSITY OF *P. MARI-NUS* INFECTIONS IN OYSTER RECOVERY AREAS IN MARYLAND.** Gustavo W. Calvo\* and Stephen J. Jordan, Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654.

To rehabilitate depleted oyster stocks, the Maryland Oyster Roundtable designated Oyster Recovery Areas (ORAs) in 6 Chesapeake Bay tributaries. We report on the current status and variability of oyster diseases in selected ORAs. Sampling to determine intra-bar and inter-bar variability of disease parameters was conducted in the Choptank River ORA in June 1994 on Cabin Creek (CRCA), Dixon/Mill Dam (CRMD), and Irish Creek (BCIC) oyster bars. Salinity was 4 ppt at CRCA, 5 ppt at CRMD, and 7 ppt at BCIC. Water temperature was 26–27°C. Oyster samples were collected with patent tongs from 35–106 grid-point stations per oyster bar. When sample size was >30, 30 oysters were haphazardly selected. A total of 1461 oysters was collected from all bars. It was not infrequent for stations to have no oysters. Oysters were bled, shucked, and tissue and hemolymph samples processed for disease diagnosis. More than 1200 samples of tissues and hemolymph were examined for *P. marinus* using Ray's fluid thioglycollate medium (RFTM) assays. Overall prevalence and intensity of *P. marinus* infections was low for CRCA samples and relatively high for BCIC samples. Overall prevalence (rectal) was 3.15% for CRCA, 1.49% for CRMD, and 88.13% for BCIC. Average ( $\pm$ SD) prevalence was 2.38% ( $\pm$ 3.83) at CRCA, 1.87% ( $\pm$ 4.27) at CRMD, and 83.42% ( $\pm$ 13.50) at BCIC. In September 1994 the sampling program was expanded to ORAs in the Chester, Nanticoke, and Severn Rivers. This study shows the importance of variability in disease prevalence between and within oyster bars; *P. marinus* prevalence can vary by a factor of >10 over a salinity gradient of only 2 ppt within a single tributary.

**SIZE-SPECIFIC FECUNDITY OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*, DURING ONE SPAWNING PERIOD IN THE MID-ATLANTIC RESOURCE AREA.** Ryan B. Carnegie and William D. DuPaul, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Knowledge of size-specific fecundity is important in estimating spawning stock biomass (SSB) of *Placopecten magellanicus*, particularly as younger age-classes are predominant in natural scallop populations. The objective of this study was to estimate size-specific fecundity of scallops from the mid-Atlantic during the spring 1993 spawning period for 4 shell height intervals (65–75 mm, 85–95 mm, 105–115 mm, and 120–130 mm) using direct counts and high resolution light microscopy (HRLM). The direct counting technique entailed the rinsing of oocytes from lacerated

ovaries ( $N = 173$ ) to form suspensions, from which were drawn aliquots for the counting of oocytes in a Sedgwick-Rafter Cell using a compound microscope (60 $\times$  mag.). Mean oocyte counts for specimens in the 4 size classes were  $2.9 \times 10^5$ ,  $1.0 \times 10^6$ ,  $2.4 \times 10^6$ , and  $3.0 \times 10^6$ , respectively. HRLM was used to validate this trend. Volume fractions were obtained for mature and atretic oocytes and other ovarian structures through point-counting, using a reticule of 64 points (60 $\times$  mag.). Fecundity was calculated ( $N = 53$ ) using the equation: Fecundity = ( $\text{mass}_{\text{ovary, wet}}(\text{g})$ ) ( $\text{VF}_{\text{mature oocytes}}$ )/ $1.6 \times 10^{-7}$ , with  $1.6 \times 10^{-7}$  representing the value for wet mass of a single oocyte used previously in determinations of fecundity for *P. magellanicus* (1,2). Using this method, mean fecundity estimates for scallops in the 4 size classes were  $6.4 \times 10^6$ ,  $6.5 \times 10^6$ ,  $1.2 \times 10^7$ , and  $1.7 \times 10^7$ , respectively. While the histologic estimates of size-specific fecundity exceeded those obtained using direct counts, the trends were comparable in terms of the factors by which oocyte production of larger scallops exceeded that of smaller scallops. This study shows that while obtaining absolute estimates of fecundity is difficult, it is possible to use direct counts and histology to quantify size-specific fecundity of sea scallops in a relative sense. These results also suggest that the SSB calculations in the Sea Scallop Fishery Management Plan (SSFMP) may be overestimated by the inclusion of 3- and 4-yr-old scallops (<105 mm).

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**EARLY GAMETOGENESIS AND SPAWNING IN THE ATLANTIC SURFLCLAM (*SPISULA SOLIDISSIMA*) FROM THE NEW JERSEY COAST.** Marnita M. Chintala\* and Judith P. Grasse, Institute of Marine and Coastal Sciences, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903.

Small surfclams collected on 1 October 1993 from a 15 m station on the continental shelf off Tuckerton, NJ were capable of spontaneous spawning and production of viable larvae when subjected to an acute increase in temperature in the laboratory. These clams were approximately 3 months old and ranged in length from 7 to 10 mm. Subsequent collections were made in late October and mid November, 1993 for histological analysis of the gonads. In October, 39% of the individuals ( $n = 89$ ) had ripening gonad material with an approximately equal sex ratio. The length of the smallest reproductive male was 3.4 mm and the smallest female was 5.1 mm. In November, 36% of the clams ( $n = 98$ ) were gametogenic with 64% of individuals male. The shell lengths of the smallest male and female clams were 5.4 mm and 5.6 mm, respectively. In October, 34% of the females and 5% of the males were partially or fully spent, while in November 16% of the females and 39% of the males were partially or fully spent. In October the largest percentage of partially spent/spent individuals

was in the 5.1–10.0 mm size class, whereas in November the largest percentage of spent clams was in the 10.1–15 mm size class. This suggests that surfclams can reach sexual maturity and spawn within 3 months of settlement. The viability and fate of the larvae produced by these spawnings is unknown.

**ANNUAL VARIATION OF HEMOLYMPH COMPONENTS AND *P. MARINUS* INFECTION IN OYSTERS SAMPLED FROM DEEP WATER SHOAL, JAMES RIVER, VIRGINIA.** Fu-Lin E. Chu\*, Aswani K. Volety, and Jerome F. La Peyre, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The seasonal variation of hemolymph components and *Perkinsus marinus* (Dermo) infection in oysters (*Crassostrea virginica*) sampled (August 1990–July 1991) from Deep Water Shoal of James River (DWS), Virginia and from oysters which were collected from the same site at the beginning of the study and maintained in the laboratory in a flow-through flume received raw York River water (YRW) were investigated. Total number of hemocytes (TC, including number of hemocytes of different sizes, ranged from 2.5 mm to 13.0 mm), percent of granulocytes (PG), serum protein (P) and lysozyme (L) of individual oysters were measured. Oysters were then diagnosed for *Perkinsus marinus* (Dermo) infection and condition index (CI) were determined. Generally, in both DWS and YRW oysters, higher TC and PG occurred in the months of higher water temperatures. L concentration was highest in the months of lower water temperatures and in the case of DWS oysters concomitantly with low salinities. P fluctuated between months, maximum P was found in DWS oysters in April and in YRW oysters in January and April. The highest condition index (CI) of DWS oysters was in the month of June and of YRW oysters was in May. *P. marinus* prevalence was greatest in DWS oysters in October (85%) and in YRW oysters in June (65%) and July (75%), the months of relatively high temperatures (23–28°C). *P. marinus* intensity coincided with infection prevalence. No infection was detected in DWS oysters in February and March, the months of low temperatures and salinities. Prevalence in DWS oysters in June (5%) and July (25%) was unusually low due to the consistent low salinity (0–3 ppt) from January to May.

**A STRATEGY FOR RESTORATION OF WHITE ABALONE, *HALIOTIS SORENSENI*.** Gary E. Davis and Peter Haaker.

The white abalone *Haliotis sorenseni*, is a deep water species occurring at depths greater than 26 m on rocky, open water reefs in southern and Baja California. White abalone was once a commercially and recreationally fished species, being highly sought after for its tender white meat. After 6 consecutive years of annual landings greater than 20 metric tons in the mid-1970s, commercial and recreational landings collapsed after 1977. These landing data indicate potentially depleted stocks. Preliminary SCUBA surveys

in 1992–1993 at the California Channel Islands confirmed that the white abalone population in that portion of southern California collapsed, possibly from recruitment failure. Only three live white abalone were found in 30,600 m<sup>2</sup> of appropriate habitat that would have supported 9,500 to 43,500 white abalone at mid-1970s densities. If white abalone population densities are similar throughout the rest of its range, the species is in danger of extinction. Currently, the California Department of Fish and Game is seeking closure of the white abalone fishery, and the National Marine Fisheries service is considering the status of the species relative to the Endangered Species Act. A successful restoration strategy will include mitigation, population assessment, captive breeding and rearing, broodstock reintroduction, and wild stock husbandry.

**AGE AND GROWTH OF *MERCENARIA MERCENARIA* IN TWO SISTERS CREEK ESTUARY, SOUTH CAROLINA.** Nathalie Devillers and Arnold G. Eversole\*, Department of Aquaculture, Fisheries and Wildlife, Clemson University, SC 29634–0362, William D. Anderson, South Carolina Department of Natural Resources, Charleston, SC 29422–2559.

*Mercenaria mercenaria* were sampled for 4 sites of an area of Two Sisters Creek which had never been commercially exploited. Shell lengths (anterior-posterior axis, SL) were measured and ages were estimated from increments in shell sections. Mean SL of hard clams collected near the mouth of the creek were significantly larger than those found at sites further upstream. The backcalculated mean SL, however, were similar among the sites. Analysis of ages revealed that hard clams near the mouth were significantly older than those collected further upstream. Differences in the age structure was also observed among sites. A recruitment hypothesis is proposed to explain the upstream pattern of decreasing sizes and ages of hard clams in Two Sisters Creek.

**BURROWING SHRIMP CONTROL AND OYSTERS IN WILLAPA BAY, WASHINGTON: DOES A PESTICIDE SOLVE THE PROBLEM?** Brett R. Dumbauld\*, Washington State Department of Fish and Wildlife, P.O. Box 190, Ocean Park, WA 98640, USA; David A. Armstrong, School of Fisheries, University of Washington, Seattle, WA 98195, John R. Skalski, Center for Quantitative Sciences, University of Washington, Seattle, WA 98195.

Experiments were conducted on the efficacy of the pesticide carbaryl, used to control the mud shrimp *Upogebia pugettensis* and ghost shrimp *Neotrypaea californiensis* on oyster culture grounds in Washington state coastal estuaries. Survival and growth of juvenile oysters and re-invasion of shrimp were monitored on 100 m<sup>2</sup> treated plots and untreated controls, as well as a larger 2.6 ha plot. Results indicate marked differences exist between the effects of each species of shrimp; ghost shrimp causing a much higher loss of oyster seed and rapidly re-invading treated areas. No significant effect of shrimp removal could be detected on oyster growth parameters including condition index.

**CONTROL OF TWO BURROWING SHRIMP SPECIES, GHOST SHRIMP, *CALLIANASSA CALIFORNIENSIS* AND MUD SHRIMP, *UPOGEBIA PUGETTENSIS*, USING SUB-SURFACE INJECTION OF CARBARYL ("SEVIN") AS AN ALTERNATIVE TO AERIAL APPLICATION IN PREPARATION OF OYSTER BEDS FOR SEEDING.** James E. Durfey\*, Washington State University, Biological Systems Engineering Department, Pullman, WA 99164-6120, James E. Durfey and John B. Simpson, University of Idaho, Fish & Wildlife Department, Moscow, ID 83843.

Following El Nino of 1982-83, a significant increase in burrowing shrimp abundance occurred affecting production for Washington State oyster growers. Environmental Protection Agency authorized aerial treatment of carbaryl over seed beds as well as bare ground on 800 acres in the state. Justification for treatment is based on general affects from burrowing shrimp to the oysters by: 1) a reduced growth rate and/or poorer condition of the oysters from feeding in competition with shrimp; 2) operational difficulties, 3) loss of seed and adults from smothering of fine sediments and sinking of shell in the softened substrate. The Environmental Impact Statement estimated a revenue loss of \$5 million and 300 jobs could result without means to control burrowing shrimp. In response to concerns about the aerial application of carbaryl into the marine environment in Wallapa Bay, WA, alternatives were explored. Washington State Department of Fisheries provided the test area which had been a producing oyster bed until burrowing shrimp recruitment and establishment no longer permitted quality habitat for oyster production. Sub-surface injection was selected, targeting the burrowing shrimp. Carbaryl was injected into 36 plots, 5' x 15'. Injection rates of 3, 6, and 9 LB per acre with row spacing of 12", 18" and 24" established a basis for effectiveness. Twenty-four hours following injection resulted in a 50% reduction of burrowing shrimp for all plots. Subsequent low tide series showed a 99% control for selected rates and row spacing. Significantly more control was achieved using the 12" row spacing as opposed to the 18" and 24" spacing for all rates. Within the measured groups using the 12" row spacing, control was achieved using 66% less chemical than aerial application. This test series suggests that subsurface injection is an effective method with a significant reduction of chemical in the environment.

**LARVAL AND JUVENILE BIVALVE MOLLUSC DISEASES—OCCURRENCE, IMPACT AND MANAGEMENT.**

Ralph A. Elston, Battelle Marine Sciences Laboratory, 1529 West Sequim Bay Road, Sequim, WA 98382.

New, unreported diseases of larval and juvenile bivalve molluscs as well as previously reported diseases have a significant impact on the intensive production of these shellfish. The pathological manifestations and occurrence of previously unreported diseases of intensively farmed larval and juvenile bivalves including idiopathic larval velar degeneration, larval shell anomalies, hemocytic hyperplasia of juvenile Pacific oysters, growth retarda-

tion of juvenile bivalves, juvenile visceral atrophy of various clams and oysters, and hemocytic neoplasia of juvenile mussels, will be reported. Each condition can have serious consequences on the growth of individual bivalves which may not recover from the diseases. Significant lesions in larval bivalves appear essentially irreversible while juvenile bivalves have increased potential for tissue repair. Exact causes of each of these diseases is not known but potential etiologies included toxic algae, bacterial exotoxins, and invasive infectious agents will be considered. Larval velar degeneration, larval shell anomalies, and juvenile visceral atrophy may result from water soluble toxins while hemocytic hyperplasia of juvenile oysters suggests an infectious etiology. The occurrence of each of these diseases observed in a diagnostic caseload will be reported and compared to occurrence, geographic location, and prevalence of previously reported diseases such as oyster velar virus disease, herpes virus of oyster larvae and juveniles, and juvenile oyster ligament disease. Approaches to management of these diseases will be presented.

**WATER QUALITY AND MANAGEMENT IN CRAWFISH CULTURE PONDS.** Arnold G. Eversole\*, Department of Aquaculture, Fisheries and Wildlife, Clemson University, SC 29634-0362, David E. Brune, Department of Agricultural and Biological Engineering, Clemson University, SC 29634-0357.

This overview will include a summary of the water suitable for culture of *Procambarus* species; a year's observations of water quality in commercial crawfish ponds (Hymel 1985); an evaluation water quality in rice forage ponds with and without crawfish (Brune and Eversole 1993); and an examination of water quality and nutrient dynamics in rice forage, rice forage with supplemental feed and total feed crawfish ponds (Brune and Eversole, unpublished data). Desirable levels of dissolved oxygen (DO) should be  $\geq 3.0$  mg/L, total hardness  $\geq 50$  mg/L, free CO<sub>2</sub> < 5.0 mg/L, iron < 3.0 mg/L and 6.5-8.5 pH. Commercial crawfish ponds in Louisiana experienced poorest water quality in November, April and May when water temperatures > 18°C. Only DO and free CO<sub>2</sub> approached levels considered stressful to crawfish. The amount and type of vegetation in commercial ponds impacted water quality. Comparison of rice-forage ponds with and without crawfish revealed an impact of crawfish activity; a depression in DO was observed in crawfish ponds as specific growth rate increased and water temperatures approached 13°C in late fall and early spring. Although nitrogen mass balance indicated that 23% of the fertilizer nitrogen was harvested as crawfish, a larger proportion was trapped in the sediment. Nitrogen fixation apparently added nitrogen to sediment because increases were in excess of fertilizer additions. Significant differences were observed in the sediment nitrogen content of ponds among the rice-forage, rice forage with supplemental feed and total feed crawfish treatments over 2 production years. The total and supplemental fed ponds received 1.9x and 1.2x more nitrogen but had 62 and 83% < nitrogen in

the sediment than the rice forage ponds, respectively. Other differences were observed in DO, nitrogen and COD of water among treatments. Our observations indicate that the nitrogen economy of rice forage crawfish ponds is different from intensive crawfish and finfish culture ponds.

**CONTROLLING POPULATIONS OF BURROWING THALASSINID SHRIMP ON OYSTER CULTURE GROUNDS: EFFECTS OF HARVESTING AND SHELL CONFIGURATION ON RECRUITMENT OF YOUNG-OF-THE-YEAR.** Kristine L. Feldman\* and David A. Armstrong, School of Fisheries, WH-10, University of Washington, Seattle, WA 98195, Brett R. Dumbauld, Washington State Department of Fish and Wildlife, Willapa Bay Field Station, P.O. Box 190, Ocean Park, WA 98640, USA; Christopher J. Langdon, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Survival and growth of the Pacific oyster *Crassostrea gigas* along the Pacific coast of the United States are adversely affected by burrowing shrimp *Neotrypaea californiensis* and *Upogebia pugettensis*. In Washington State (USA), the insecticide carbaryl has been applied to oyster beds to control populations of shrimp for more than 30 years. While carbaryl removes adults from oyster grounds, it does not discourage reinvasion by young-of-the-year (YOY) shrimp. We initiated a study to examine how the harvest and culture of oysters affects recruitment success of burrowing shrimp. Young-of-the-year abundance was quantified prior to and 2 wks after dredging a mature oyster bed to assess whether active methods of disturbance, such as harvest operations, might kill YOY shrimp residing in shallow burrows. We found no conclusive evidence, however, that dredging had a significant impact on shrimp density distinct from natural mortality. Young-of-the-year shrimp were also quantified in areas of oyster culture and dense epibenthic shell cover to determine if shell configuration (i.e., structure and percent cover of shell) affects recruitment success. Mean densities of *N. californiensis* were significantly lower in a mature oyster bed and an area of dense epibenthic shell than in a seed bed and an open mudflat. Results of field and laboratory experiments indicate that epibenthic shell hinders recruitment of *N. californiensis* by acting as a physical barrier to settlement and harboring high densities of YOY Dungeness crab, *Cancer magister*, which prey on newly settled shrimp. In contrast to *N. californiensis*, however, mean densities of YOY *U. pugettensis* were significantly higher in epibenthic shell habitats than open mudflat; mechanisms underlying their patterns of distribution currently are being investigated. It is hoped that information on shrimp recruitment will aid in developing an integrated pest management plan to achieve more effective long-term management of shrimp populations in areas of bivalve culture.

**SETTLEMENT AND EARLY GROWTH OF THE SCALLOP *ARGOPECTEN CIRCULARIS* IN BAHIA CONCEPCION, BAJA CALIFORNIA SUR, MÉXICO.** E. F. Felix-Pico\*, A. Tripp-Quezada, J. Castro-Ortiz and G. Serrano-casillas, CICIMAR-IPN, Apdo. Postal 592, La Paz, B.C.S., 23000 México \*Scholarship from COFAA-IPN and Desempeno Académico (SEP), G. Bojorquez-Verastica and Y. G. Lopez-Garcia, Secretaria de Pesca, Dir. Acuicultura, Ocampo y Lic. Verdad S/N, La Paz, B.C.S., 23000 México.

Research on scallop population in Bahia Concepcion has as its objectives: 1) to determine the intensity and duration of spatfall on artificial collectors, 2) determine a means to keep the spat enclosed for 4 months until they are ready for release and repopulation. During the 7 years of this study, the major scallop spatfall was observed to occur during January to March, Spatfall one year can therefore be related to recruitment in the next with years of high and low spatfall reflected in subsequent differences in recruitment. For Bahia Concepcion in 1987, the settlement resulting from a reduced winter-spring spawning was 500 spat per bag. The total stock was estimated to be 70 million scallops. For 1988, the settlement increased to 40,000 spat/bag and the stock was estimated at 120 million scallops. For 1991, the settlement decreased to 18,000 spat/bag and the stock was about 50 million scallops. For 1994, the settlement was very low, with only about 157 spat/bag, and the stock decreased to 1 million scallops. Weekly data for 1994, showed variation between 25.3 to 358.7 spat/bag. The final size of the scallops held for 4 months in an enclosure will be 4.5 to 5.2 mm when released.

**EXPOSURE OF EASTERN OYSTERS TO TRIBUTYL TIN INCREASES THE SEVERITY OF *PERKINSUS MARINUS* DISEASE.** William S. Fisher\*, U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, FL 32561, Leah M. Oliver, Avanti Corp., c/o USEPA, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, FL 32561, Erin B. Sutton, Gulf Coast Research Laboratory, 703 East Beach Dr., Ocean Springs MS 39566, USA, C. Steve Manning, and William W. Walker.

Oysters (*Crassostrea virginica*) that were free of the protozoan pathogen *Perkinsus marinus* were exposed in a continuous-flow system to 0, 30 and 80 ppb tributyltin oxide (TBT), the active ingredient of many marine antifouling paints. Less than 5% mortalities were recorded during the 2-month exposure period for all treatments. After exposure, oysters from each treatment were subdivided into two groups (43-44 each): An inoculum of oyster tissue infected with *P. marinus* was added to the challenge group (CH) and an autoclaved inoculum of the same tissue was added to the unchallenged group (UN). Oysters were maintained in 6

aquaria at 21 ppt salinity and 25°C temperature and fed an algal mixture daily. After 9 weeks, oyster survival for UN groups was 93.0, 93.2 and 62.8% for 0, 30 and 80 pptr TBT treatments, respectively, indicating some TBT-related mortality at the highest concentration. In the CH groups, survival was 88.6, 50.0 and 44.2%, respectively, demonstrating greater mortality with increased TBT concentrations. Whole-body diagnosis for *P. marinus* performed in the blind showed CH groups had higher prevalence and greater numbers of *P. marinus* than UN groups at the same treatment. The greatest differences were observed in the 30 pptr TBT treatment: Prevalence for CH was 100% with an average intensity of 194,698 *P. marinus* per oyster and prevalence for UN was 63%, but with an average intensity of only 17 per oyster. The CH group for 0 pptr TBT exhibited 88% prevalence, but intensity was only 1/10 that of the 30 pptr CH group (18,785 per oyster). These data indicate that exposure of oysters to TBT can increase the prevalence and intensity of *P. marinus* in challenged oysters and that these 2 stresses cause greater mortalities in combination than either singly.

**A COMPARISON OF FIELD NURSERY METHODS FOR THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*, IN COASTAL NEW JERSEY ESTUARIES.** Gef Flimlin and John Kraeuter, New Jersey Sea Grant Marine Advisory Service, 1623 Whitesville Road, Toms River, NJ 08755; Haskin Shellfish Research Laboratory, Rutgers University, Institute of Marine and Coastal Sciences, Box B-8, Port Norris, NJ 08349.

The initial capital investment for hard clam seed for the shellfish aquaculturist can sometimes be a significant burden at the beginning of a growing season. In order to examine the potential for buying smaller clam seed from a hatchery and performing the nursery phase in the estuary, 5 stocking densities were examined in on-bottom and off-bottom plastic mesh bag nursery systems. Stocking densities ranged from 2000 to 6000 5 mm hard clam seed. A second experiment examined 3 nursery containers, an on-bottom plastic mesh bag, an off-bottom plastic mesh bag, and a tented on-bottom soft nylon mesh bag. This also used 5 mm seed. In the first trial, the off-bottom bags showed no consistent pattern with density; the on-bottom had significant differences from expected, but no consistent pattern indicating that the density had anything to do with the growth to plantable size. In the second trial, the results were also inconsistent with the expected, and was redone with a better sampling technique. Mortality rates over both trials were very low, and all shellfish were eventually field planted for commercial growout.

**HAPLOSPORIDIAN INFECTIONS OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* THUNBERG.** Carolyn S. Friedman\*, California Department of Fish and Game, c/o Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA 94923, Ronald P. Hedrick, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis CA 95616.

Haplosporidian infections were observed in adult and seed Pacific oysters, *Crassostrea gigas* Thunberg, from Matsushima Bay, Japan in 1989 and 1990 (Friedman and Hedrick 1991, Friedman et al. 1991). Multinucleated plasmodia and/or spore stages were observed in 2-13% of the oysters examined from Japan. Parasite morphology, tissue specificity and synchrony of sporulation closely resembled those of *Haplosporidium nelsoni*, the causative agent of Delaware Bay Disease of the American oyster, *C. virginica* Gmelin.

Pacific oysters from Matsushima Bay, Japan, had been imported into Drakes Estero, CA and Grays Harbor, WA, for several years. We sampled domestic Pacific oysters from three embayments in California and 2 locations in Washington state between 1990 and 1993. Oysters were also collected from Matsushima and Watanoha Bays, Japan. One to three percent of the Pacific oysters from Drakes Estero had mild systemic or localized infections with haplosporidia. Plasmodia were observed within the gills and connective tissues (VCT) surrounding the stomach or intestine or, more commonly, within the epithelium of the heart. An inflammatory response was observed in response to plasmodia within VCT; inflammation was not observed in infections within the heart. No haplosporidia were observed in oysters from Tomales and Humboldt Bays in California or Grays Harbor, Washington. A single oyster from the Hood Canal at Quilcene had a mild infection that consisted of multinucleated haplosporidia-like plasmodia and host hemocytes surrounding the intestine. No haplosporidia were observed in oysters from Quilcene in later samples. Multinucleated plasmodia were observed in seed oysters from Watanoha Bay, Japan. Thus, haplosporidia are not limited to Pacific oysters grown in Matsushima Bay, Japan; these protozoans are established at very low levels in domestic stocks of Pacific oysters reared in Drakes Estero, California, and may be present in those grown in Washington state.

**THE USE OF GYNOGENESIS IN MAPPING OF ALLOZYME LOCI IN THE DWARF SURFCLAM, *MULINIA LATERALIS* SAY.** Ximing Guo and Standish K. Allen\*, Jr., Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, B-8, Port Norris, NJ 08349.

Genetic maps are useful for marker assisted selection, direct manipulation of genes, analysis of non-random association of genes, and phylogenetic comparisons. Because gynogenesis can be used to estimate gene-centromere distance, it is very helpful in determining gene order of linked loci using centromere (C) as an anchor point. We used gynogenesis along with family analysis to map allozyme loci in the dwarf surfclam, *Mulinia lateralis* Say. Eggs from females were divided into 2 groups: one was used to

make a normal full-sib family, and the other was used to produce meiotic gynogens. For gynogenesis, eggs were fertilized with ultraviolet light-irradiated sperm and subsequently treated with cytochalasin B to block the release of the second polar body (PB2). Three replicates were made. The parents and clams from the 2 progeny groups were screened at 21 allozymic loci, and 15 loci were polymorphic in the 3 replicates. Of all gynogens analyzed, no paternal alleles were observed at all diagnostic loci suggesting that the gynogenetic procedure was very effective. Three linkage groups were identified: AAT = GPI-C, PGDH-FH-C, and TAPI-DAP-IDH1-C. The use of gynogenesis successfully determined the gene order of linkage groups respective to their centromeres. Analysis of gene-centromere distances revealed that several loci (AAT, GPI, PGDH, TAPI and PGM had a recombinant frequency of 90-100% indicating strong or complete interference.

**TETRAPLOID PACIFIC OYSTERS (*CRASSOSTREA GIGAS* THUNBERG) HAVE HIGH FECUNDITY DESPITE MULTIVALENT FORMATION DURING MEIOSIS.** Ximing Guo\* and Standish K. Allen, Jr., Haskin Shellfish Research Laboratory, Institute of Marine and Coastal Sciences, Rutgers University, B-8, Port Norris, NJ 08349.

Triploid mollusks are useful for aquaculture because of their sterility and improved growth. Interests in tetraploids have been based on the assumption that tetraploid mollusks are fertile and can be used for the production of triploids. We successfully produced viable tetraploid Pacific oysters by blocking polar body I in eggs from triploids (*Mol. Mar. Biol. Biotechnol.* 3:42-50). Here we present our preliminary observations on the reproductive biology of tetraploid Pacific oysters. Like normal diploids, tetraploid oysters matured at 1 year of age. At maturation, tetraploids sampled were either female or male with an approximately normal sex ratio. In contrast to triploids whose gonads are visibly reduced, tetraploids exhibited gonads that were as developed as those of normal diploids in appearance. The fecundity of tetraploid females ranged from 1.4 to 4.2 million, which was not significantly different from that of normal diploid females, ranging from 2.0 to 3.3 million. Eggs produced by tetraploids were 70-80% larger than those from normal diploids in volume ( $p < 0.001$ ). Large eggs may lead to improved larval growth. Chromosome analysis of eggs from tetraploids revealed the prevailing formation of multivalents during meiosis. The formation of multivalents in triploids has been commonly regarded as the cause for sterility. The fact that tetraploids had high fecundity despite the prevalence of multivalents suggests multivalent formation is not inhibitory to fecundity. Flow cytometry analysis showed that sperm from tetraploids had a diploid DNA content. Those results suggest that tetraploids may provide an effective method for triploid production. Cross matings between tetraploids and diploids are being tested in our lab.

**WITHERING SYNDROME IN CALIFORNIA: DOES IT OCCUR IN SUBTIDAL ABALONES?** Peter L. Haaker\*, Scott L. Harris and Ian K. Taniguchi, California Department of Fish and Game, 330 Golden Shore, Suite 50, Long Beach, CA 90802, Carolyn S. Friedman, California Department of Fish and Game, Bodega Marine Laboratory, Bodega, CA 94923.

Withering syndrome (WS) is a lethal disease-like affliction of the intertidal black abalone (*Haliotis cracherodii*) that causes atrophy, weakness, and tissue discoloration. It was first reported at 3 California Channel Islands in 1985, and has since spread throughout the islands. Black abalone populations had been extirpated from six of eight Channel Islands by 1992, and the fishery for that species was closed in 1993.

Red (*H. rufescens*), green (*H. fulgens*), pink (*H. corrugata*), and white (*H. sorenseni*) abalones overlap black abalone distributions in southern California, and red and green may occur in close association with black abalone. In dense populations of the intertidal black abalone WS was hard to miss, but in low density populations of subtidal abalones, WS is more difficult to assess. Here we report on WS-like symptoms in subtidal abalones and discuss the implications for continued fisheries.

**MICROHABITAT SELECTION AT SETTLEMENT BY PLANKTONIC SCALLOP LARVAE: ACTIVE VS PASSIVE PROCESSES.** Michel Harvey\* and Edwin Bourget, GIROQ and OPEN (Ocean Production Enhancement Network), Département de biologie, Université Laval, Ste-Foy (Québec), Canada, GIK 7P4.

Sampling carried out in the Baie des Chaleurs (Québec, Canada) in 1990 and 1991 showed that newly settled Iceland scallop spat (*Chlamys islandica*) were not randomly distributed on the sea bottom. Various inorganic and organic substrata were examined. Twenty times more postlarvae were found on perisarc of the dead hydroid, *Tubularia larynx*, than on any other natural substrata. Both laboratory and field experiments were carried out to assess the mechanisms involved in the association between spat of the Iceland scallop and dead hydroids. Results suggest that the greater number of Iceland scallop spat found on dead hydroids results from a combination of both passive and active processes acting at different passively until contact with a filamentous epibenthic structure where their probability to come into contact with different parts of the structure depends on the spatial arrangement (branch diameter and heterogeneity) of the branches within the structure. On the other hand, small scale processes would involve active micro-habitat evaluation based on the chemical nature of the chitinous perisarc of hydroids.

**GENETICS AND BROODSTOCK MANAGEMENT.** Dennis Hedgecock, University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923.

Broodstock management means many things to many people. Here I focus on the management of broodstock for the purposes of maintaining genetic diversity and providing the basis for genetic improvement programs. Management for purposes of genetic improvement is quite distinct from management of broodstock for hatchery production of seed. Indeed, separate hatchery and broodstock facilities, for genetic improvement and for seed production, may often be necessary.

Broodstock management for genetic improvement is only possible when stocks are hatchery-propagated with little or no input of individuals from the wild, i.e., genetic improvement can only be done on closed populations. Closed populations of finite size, however, tend to lose genetic diversity and become inbred by the sampling process known as genetic drift. Unfortunately, studies of aquatic hatchery broodstock have revealed substantial genetic drift to be the rule rather than the exception. Fourteen of 17 estimates of effective population size, based on measurements of genetic drift in hatchery stocks, are less than 50, 9 are less than 25. This drift is likely the result of inadequate numbers of broodstock and, more importantly, large variance in the reproductive success of individual broodstock. While small population size is tolerable and even useful in breeding domesticated livestock, genetic drift in small populations of undomesticated stocks of shellfish may greatly reduce the chances of domesticating improved varieties in the future. Moreover, we may not always have the luxury of reserving genetic drift and inbreeding trends by collecting new wild broodstocks.

There are simple, non-technical steps that can be made in the husbandry of broodstock to alleviate the causes of genetic drift. The most important may be the dissociation of breeding and seed production operations; from this follows the next most important step, establishing some level of control over pedigree and the reproductive contributions of individuals, information typically lost in mass spawnings for seed production. Alternatively, the expensive technology of DNA fingerprinting can be used to obtain pedigree information on the best performers from hatchery mass spawns, a process that has been termed "walk-back selection". Still, there may be need to establish a population of such selected brood stock independently of the commercial seed-production brood stock.

Loss of genetic diversity to random drift can be measured by studies of marker loci, such as allozymes and microsatellites. Yet, genetic diversity in production traits is what is important to improvement. Current university-based research is beginning to obtain information on components of variance and heritabilities for production traits, primarily growth rate. This research can help guide the design of commercial breeding programs, which can eventually monitor the relevant genetic diversity by responses to selection and reproductive performance.

**DEVELOPMENT OF SPECIFIC MOLECULAR PROBES FOR SEROLOGICAL AND PCR ASSAYS FOR THE IDENTIFICATION AND DIAGNOSIS OF *MIKROCYTOS MACKINI*, THE CAUSE OF DENMAN ISLAND DISEASE IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*.** Dominique Hervio\*, Gary R. Meyer, Susan M. Bower, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC, V9R 5K6, Canada, Robert D. Adlard, Department of Parasitology, University of Queensland, Brisbane, QLD, 4072, Australia.

"Microcell" parasites in the genera *Bonamia* and *Mikrocytos* are associated with diseases in oysters. As a result of the presence of haplosporosomes in these protozoa, it has been suggested that they both belong to the phylum Ascomycota. Polyclonal and monoclonal antibodies against *M. mackini* were obtained and are being characterized. They will be tested against isolates of *M. mackini* from Pacific oysters, *Crassostrea gigas*, (British Columbia, Canada) and *M. roughleyi* from Sydney rock oysters, *Saccostrea commercialis*, (New South Wales, Australia) and used to compare the antigenic characteristics of the two species—PCR amplification of rDNA using primers in conserved regions was conducted for isolates of *M. mackini*. The *M. mackini* amplified nucleotide sequence will be compared to that of *M. roughleyi*. The antibodies or PCR amplification will be used for pathogen detection and disease diagnosis. Analysis of the nucleotide sequence indicated that *M. mackini* is not homologous to those of *Bonamia* spp. A recent electron microscopy study showed that *M. mackini* lacks mitochondria. These differences indicate that *M. mackini* and *Bonamia* spp are not related.

**THE HATCHERY PRODUCTION OF THE ALASKAN LITLENECK CLAM (*PROTHACA STAMINEA*).** Jeff Hetrick, Alaska Shellfish Development Corporation, P.O. Box 2643, Seward, AK 99664.

Adult littleneck clams *Protothaca staminea* from Tatitlek, Prince William Sound were transported to the Qutechak Shellfish Hatchery in Seward in January 1994. Four broodstock families of 15 individuals each were conditioned in mildly aerated 69 L tanks at 16–18°C and fed mixtures of *Tahitian isochrysis* and *Chaetoceros calcitrans*. Maturation was monitored biweekly by dissecting gonadal tissue. The first spawning event occurred, without inducement, on 22 February 1994. Spawnings occurred throughout the summer at approximately two week intervals. Larvae were cultured in 60 L tanks. When the clam reached approximately 240 µm at day 26 they were transferred to a 130 L airlift system on the downwelling mode. Larvae were fed a mixture of *T. iso*, *C. calcitrans* and *Thalassiosira pseudonana* twice a day at approximately 70,000 cells/mL per feeding. *Tetraselmis suecica* was added to the mix after the sixth spawn. The temperature, setting substrate, feed ration, feed composition, time of placement into the downweller and frequency of changing out the water were altered with each cohort until the clams successfully metamorphosed after the sixth spawning. Successful setting occurred with

a combination of fully mature gametes, 16-18°C and 22 ppt filtered (2 µm) seawater, three day cycle on changing the water, transfer to airlift system when the clams to become sessile and the addition of *T. suecica* to the algae mix. Presently, 6 cohorts (approximately 2,000,000 are under culture at 1-3 mm. Unusual preset and postset behavior has been observed.

**DISCRIMINATION BETWEEN TWO CLOSELY RELATED SPECIES OF OYSTERS, *CRASSOSTREA VIRGINICA* AND *C. RHIZOPHORAE*, USING NUCLEOTIDE SEQUENCE VARIATION OF THE MITOCHONDRIAL CYTOCHROME OXIDASE I GENE.** Adam W. Hrinevich\*, David W. Foltz and Ya-Ping Hu, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

The purpose of this study was to characterize the nucleotide sequence variation in the mitochondrial cytochrome oxidase I (CO-I) gene between 2 closely related and morphologically similar oysters, *Crassostrea virginica* and *C. rhizophorae* sampled from the northern Gulf of México and Puerto Rico, respectively. In turn, these sequence differences were then used as a diagnostic tool to identify adult or larval *Crassostrea* oysters. Historically, there has been considerable disagreement by various authorities on whether there are 1 or 2 coastal species of *Crassostrea* in the north and central Atlantic. Both *C. virginica* and *C. rhizophorae* have distinct zones of distribution, with a putative contact zone along a 300-mile stretch of the southern Bay of Campeche. To examine nucleotide sequence variation, approximately 700 base pairs of the CO-I gene from both species was amplified using the polymerase chain reaction, and 219 base pairs were sequenced. Although each species was shown to have a fixed haplotype for the DNA sequence under study, there were 24 nucleotide sequence differences detected. Of these differences 88% were third position changes, none of which resulted in a change in the amino acid sequence. There was a notable bias in transition events (75%) as opposed to transversion events (25%). Additionally, upon digestion of the amplified CO-I gene with the restriction endonucleases *Spe* I and *Mlu* I, we were able to positively identify individuals from *C. rhizophorae* and *C. virginica*, respectively, based on their unique restriction fragment length polymorphisms. These enzymes can therefore be utilized as diagnostic tools to rapidly and accurately identify adult and larval *Crassostrea* oyster from the Atlantic coast of North America.

**MARYLAND OYSTER GEOGRAPHICAL INFORMATION SYSTEM: MANAGEMENT AND SCIENTIFIC APPLICATIONS.** S. J. Jordan, K. Greenhawk and G. F. Smith, Maryland Department of Natural Resources, Cooperative Oxford Laboratory, Oxford, MD 21654.

A microcomputer geographical information system (GIS) has been developed to manage and interpret data from Maryland's oyster monitoring and management programs. The GIS was initiated to portray annual monitoring information geographically, but has been expanded to include physical and chemical habitat data, management-related information, and data from special studies. Complete biological and physical information about an individual oyster bar, a region, or the entire Maryland Chesapeake Bay can be retrieved to a user's specification almost instantaneously, and portrayed in a variety of graphical and tabular formats. The system has proved especially useful in supporting the information needs of the state's Oyster Recovery Action Plan. For example, we have provided managers, scientists, and policy-makers with clear, graphical portrayals of oyster habitat, population and disease status, salinity gradients, and management history with a minimum of effort. As new experimental management efforts develop, the GIS is being used to maintain a standard, geographically precise database for documenting and tracking their performance. The use of GIS with biological monitoring data greatly simplifies the spatial aspects of analysis, allowing the analyst to focus on temporal variations: the GIS is being used to test hypotheses about historical changes in the areal extent of oyster physical habitat, spatfall and diseases. Besides its utility for management and scientific investigations, the GIS has proved to be a valuable educational tool for students and tour groups.

**PHYSIOLOGICAL ALTERATIONS OF THE BLACK ABALONE, *HALIOTIS CRACHERODII* LEACH, WITH WITHERING SYNDROME.** G. Kismohandaka, W. Roberts, R. P. Hedrick and C. S. Friedman\*, University of California, Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA 94923.

Population densities of black abalone, *Haliotis cracherodii* Leach, have steadily declined on the California Channel Islands and numerous mainland locations in southern California beginning in 1985. Mortality has been attributed to a terminal disease called withering syndrome (WS) in which abalone become weak, lethargic and emaciated. The cause of WS has not been identified. We have initiated an investigation of physiological parameters, including oxygen consumption, ammonia excretion and food consumption, of black abalone with and without WS in order to identify which systems may be affected by this serious disease. Abalone were collected from locations where WS occurs and those where

WS has not been observed during the summer and fall of 1992 and spring of 1993. All physiological parameters measured did not differ between sampling periods for each location sampled ( $p > 0.100$ ). Results suggested that weight specific oxygen consumption exists in abalone with and without WS ( $p = 0.000$ ) and that oxygen consumption rates did not differ between these 2 groups ( $p = 0.476$ ). Mass specific ammonia excretion was observed in abalone with WS in the summer and fall of 1992. During these times, ammonia excretion rates per gram body mass (wet and dry weight) were significantly different between healthy and sick black abalone ( $p < 0.001$ ).

Although ammonia excretion rates were independent of weight in healthy ( $p = 0.059$ ) and diseased ( $p = 0.156$ ) in spring of 1993, excretion rates of abalone with WS tended to increase with increasing body mass. Rates of food consumption were not significantly different between abalone with and without WS ( $p > 0.100$ ,  $p = 0.107$ ). Microscopic examination of stained tissue sections revealed that abalone with WS deplete foot muscle glycogen before muscle degeneration was observed. Severe foot muscle fiber depletion was consistently observed in abalone in advanced stages of the disease. These data suggest that abalone with WS may be using foot muscle protein as an energy source.

**SPERM PRODUCTION IN TETRAPLOID MUSSELS, *MYTILUS GALLOPROVINCIALIS*.** Akira Komaru and Katsuhiko T. Wada, National Research Institute of Aquaculture, Nansei, Mie 516-01 Japan, John Scarpa\*, Harbor Branch Oceanographic Institution, Inc., 5600 U.S. 1 North, Ft. Pierce, FL 34951.

The induction of triploidy in bivalves, especially oysters, is the most common genetic modification used in bivalve aquaculture. Unfortunately, methods for inducing triploidy do not always produce a 100% triploid population. The production of tetraploids has been suggested as one method to ensure 100% triploidy since it is expected that tetraploid individuals would produce gametes containing a diploid genome. These could be combined with normal haploid gametes to produce triploids as has been demonstrated with fish. The viability of tetraploid bivalves was first demonstrated in the mussel, *Mytilus galloprovincialis* (Scarpa et al. 1993. *Nippon Suisan Gakkaishi*, 59:2017–2023). Tetraploidy was induced by polar body inhibition with cytochalasin B. Non-random samples were taken near the end of the spawning season (March to April) from this cohort of mussels approximately one year in age. Histological preparations of gonadal material from the sampled individuals were examined by light and transmission electron microscopy. Of the 16 tetraploids sampled, four were found to be mature males, 11 were immature males, and one was her-

maphrodite. Sperm in tetraploids contained approximately twice the amount of DNA compared to sperm from diploids (measured by DNA microfluorometry) and had a mean  $\pm$  s.d. acrosome length of  $4.4 \pm 0.62$   $\mu$ m, nuclear length of  $2.04 \pm 0.05$   $\mu$ m, nuclear width of  $2.14 \pm 0.06$   $\mu$ m, flagellar length of  $72.3 \pm 2.25$   $\mu$ m. All of the measured parameters were larger compared to sperm in diploids: acrosome length ( $2.85 \pm 0.14$ ), nuclear length ( $1.85 \pm 0.06$ ), nuclear width ( $1.78 \pm 0.07$ ) and flagellar length ( $60.55 \pm 1.95$ ). From these characteristics it is anticipated that sperm from tetraploids will be viable and capable of fertilization for producing polyploid progeny.

**GROWTH AND SURVIVAL OF THE TROPICAL SCALLOP *NODYPECTEN (LYROPECTEN) NODOSUS* IN SUSPENDED CULTURE AT THREE DIFFERENT DEPTHS.** César Lodeiros\*, and John Himmelman, Département de biologie and GIROQ (Groupe interuniversitaire de recherches océanographiques de Québec), Université Laval, Québec, Canada G1K 7P4, Jesus Rangel, Luis Freite, Francisco Morales, and Anibal Vélez, Departamento Biología Pesquera, Instituto Oceanográfico de Venezuela, Universidad de Oriente, Cumaná 6101, Venezuela.

As part of an evaluation of the aquaculture potential of various Caribbean bivalves, we examined the growth and survival of *Nodypecten (Lyropecten) nodosus* which were placed in December 1, 1993 in pearl nets at 3 depths at Turpialito in the Golfo de Cariaco, Venezuela. The initial size was at 9.4 mm (SD = 1.3) in shell height. For each depth, temperature was recorded continuously using thermographs and seston, chlorophyll *a* and salinity were measured at weekly intervals. The data up to April 1993 show an almost linear increase in shell height, but the rate decreased with depth (10.0, 8.5 and 5.0 mm month<sup>-1</sup> at 8, 21 and 34 m in depth, respectively). Those at 8 m attained 51.6 mm (SD = 4.7) in April, after 4 months of culture. The rates of increase in the muscle and remaining tissues, and in shell mass, were slow during the first month but increased markedly thereafter. The rates of increase of these mass parameters were greatest at 8 m and least at 34 m. The wet mass of the muscle attained 3.4 g in April at 8 m, equivalent to the commercial muscle size for *Argopecten gibbus*. Gonadal development was most advanced at 8 m, where 90% of the scallops had gonads in February and 100% in March. Gonads were absent for scallops at 34 m in April. Monthly survival rates were consistently >80%, except at 34 m in January (63%). Mortality decreased with depth. Mean chlorophyll *a* concentrations decreased from 4.8 mg L<sup>-1</sup> at 8 m to 0.7 mg L<sup>-1</sup> at 34 m, whereas mean temperatures were similar over the depth gradient studied. This suggests that the accelerated growth at the shallower depths is more likely related to food availability than temperature.

**SCALLOP FEEDING BEHAVIOR AND EMPIRICAL MODELS.** Bruce A. MacDonald\*, Biology Department, University of New Brunswick, Saint John, NB, E2L 4L5, Canada, J. Evan Ward, Department of Biological Sciences, Salisbury State University, Salisbury, MD 21801, Jon Grant and Craig W. Emerson, Department of Oceanography, Dalhousie University, Halifax, NS, B3H 4J1 Canada, Greg S. Bacon, Department of Fisheries and Oceans, Moncton, NB, E1C 9B6, Canada.

In order to estimate realistically the carrying capacity of various coastal environments for scallops, we need to have a good understanding of the relationships between environmental conditions and the factors that determine production. This includes the influence of oceanographic conditions on the abundance, quality and delivery rates of suspended food particles, and the bivalve's physiological compensation in rates of particle utilization and efficiencies of food conversion to body tissue. The objective of this study was to establish the relationships between the food supply and feeding activity in order to predict the energy available for growth in the sea scallop *Placopecten magellanicus*. This was accomplished through a series of physiological experiments under natural food conditions, and laboratory conditions where the concentration and quality of the diet were manipulated. We found that the physiological variables indicative of energy expenditure (respiration and excretion) were conservative in their response to fluctuating food conditions while those variables important for energy gain (e.g. feeding rate and absorption efficiency) were more variable. The wide variety of adaptive feeding behaviors exhibited by bivalves, including preferential selection of nutritious particles, need to be considered when modeling physiological energetics. The use of empirical models has begun to reveal the predictive hierarchy of numerous factors important to scallop growth and will assist in the development of more general simulation models.

**RECRUITMENT IN FLORIDA POPULATIONS OF THE SOUTHERN BAY SCALLOP (*ARGOPECTEN IRRADIANS CONCENTRICUS*) AND ITS RELATIONSHIP TO ADULT DENSITY.** Dan C. Marelli\*, William S. Arnold and Catherine P. Bray, Florida Marine Research Institute, 100 8th Avenue SE, St. Petersburg, FL 33701.

Many bay scallop populations in Florida appear to be in a state of decline. Although the causes of this decline are incompletely understood, many factors from habitat degradation to overharvesting have been implicated. Recruitment is one aspect of scallop life history that may be limiting to Florida populations, particularly because the bay scallop is semelparous. Studies of recruitment may provide a general estimate of the viability of an individual scallop population. Therefore a significant portion of our research

effort is being directed to understanding the relationship between adult density and recruitment. Data on recruitment in a formerly very large but currently decimated population suggest a strong correlation between adult density and recruitment. Recruitment monitoring is continuing in consistently high density and historically high density populations that may be in early stages of recovery, and we are also monitoring recruitment in a declining population. The relationships among spawner density, recruitment, and the maintenance of "healthy" bay scallop populations in Florida are discussed from a management perspective.

**PARASITE-SPECIFIC PCR AMPLIFICATION OF AN INTERGENIC mtDNA DOMAIN OF *PERKINSUS MARINUS* IN OYSTER HEMOLYMPH: A RAPID AND SENSITIVE ASSAY FOR INFECTION SCREENING.** Adam G. Marsh and Gerardo R. Vasta\*, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 600 East Lombard Street, Baltimore, MD 21202.

The eastern oyster pathogen *Perkinsus marinus* threatens the economic viability of the oyster fishery along the Gulf of México and southern to mid-Atlantic coast. Our work in developing cDNA and genomic DNA libraries from axenically cultured *P. marinus* has provided the basic genetic information necessary to develop a highly sensitive, pathogen-specific PCR assay for the detection of *P. marinus* infections in the hemolymph of oysters sampled in the field. Specifically, we have cloned and sequenced 8.5 Kbp of the *P. marinus* mitochondrial genome (mtDNA) and have identified several non-coding intergenic domains that are ideally suited for a species-specific PCR assay. Our assay involves removing 1 ml of hemolymph from an oyster, extracting the DNA from the hemocytes, and then using 1 µg of that DNA as a template in a stringent PCR amplification (61°C annealing temperature). With this assay, we can detect as little as 10 pg of total *P. marinus* DNA per µg of oyster hemocyte DNA with EtBr staining of an agarose gel, and as little as 100 fg of total *P. marinus* DNA per µg of oyster hemocyte DNA with Southern-Blot autoradiography. Our assay has been ground-truthed using the traditional thioglycollate assay and in general is faster (requiring only 8 hours for EtBr visualization and 2 days for autoradiography) and more sensitive. There is no cross-reactivity between our PCR primers and the DNA of oysters collected from the Gulf of México and mid-Atlantic coast. We are currently developing a PCR-based assay that will allow us to rigorously quantitate the specific infection level (amount of *P. marinus* DNA) in an oyster hemolymph sample. [Supported by Cooperative Agreement No NA47FL-0163, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Oyster Disease Research Program to G.R.V.]

**CLONING AND SEQUENCE ANALYSIS OF AUTHENTIC 5S AND 16S RIBOSOMAL RNA GENES FROM A GENOMIC LIBRARY OF THE OYSTER PARASITE *PERKINUS MARINUS*.** Adam G. Marsh and Gerardo R. Vasta\*, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 600 East Lombard Street, Baltimore, MD 21202, Giacomo Bernardi, Department of Biological Sciences, University of Santa Cruz, Santa Cruz, CA 95064.

Recently, 2 separate laboratories have reported phylogenetic analyses of *Perkinsus marinus* using PCR derived sequences of the small subunit ribosomal RNA gene (Goggin and Barker 93, Mol. Biochem. Parasitol. 60:65–70; Fong et al., 93 Mol. Marine Biol. Biotech. 2:346–350). We have cloned and sequenced a 3.2 Kbp mtDNA fragment of *P. marinus* that contains the 5S ribosomal RNA gene. From this sequence information we have produced separate phylogenetic analyses utilizing both ribosomal gene sequences using a parsimonious approach with boot-strap replication (2,000) to evaluate lineage significance. These analyses are in agreement with previous studies that describe a closer affinity for *P. marinus* and dinoflagellates than exists between *P. marinus* and other members of the Apicomplexa. This observation is intriguing from two perspectives: First, almost all apicomplexans are strict intracellular parasites while dinoflagellates exist as free-living or symbiotic species, and intra- or extracellular parasites of marine organisms; thus, these two groups may have been derived from a common generalist group that has diverged based on the free-living or extracellular vs. intracellular mode of parasitic specialization. The rRNA sequence similarity between *P. marinus* and dinoflagellates is functionally supported by the fact that the trophozoite is not a strict intracellular form because proliferating trophozoites can be found in oyster plasma and moreover, *P. marinus* and actively replicate *in vitro* in the absence of host cells. Second, because of the high affinity between *P. marinus* and other dinoflagellates, research efforts to control *P. marinus* infection and virulence in oysters should consider both dinoflagellate and apicomplexan models. [Supported by Cooperative Agreement no. NA47FL-0163, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Oyster Disease Research Program to G.R.V.]

**SOME OBSERVATIONS ON ARK SHELL CLAMS, *NOETIA PONDEROSA*, AND *ANADARA OVALIS*, AND IMPLICATIONS FOR FISHERIES MANAGEMENT.** Kay A. McGraw\*, Biology Dept., Radford University, Radford VA 24141, Michael Castagna, Virginia Institute of Marine Science, Wachapreague, VA 23480.

Two species of arkshell or "blood" clams, *Noetia ponderosa* (ponderous ark) and *Anadara ovalis* (blood ark), have been harvested by watermen on the Eastern Shore in Virginia since 1991. Long considered a useless incidental catch in the harvest of hard clams and oysters, the arkshell clams now constitute a rapidly growing fishery with potential for future development. However, the paucity of information on the life history of these species in Virginia waters, coupled with intensive harvesting, presents a

problem for management of the fishery. Our study focused on growth rates and age-size relationships for both species, as well as size-frequency distributions in fisheries and non-fisheries samples. Approximately 500 juvenile *N. ponderosa* and *A. ovalis* were measured and placed in plastic grow-out trays in October 1992, and monitored for growth during the next twenty months. Results showed that *A. ovalis* grows about twice as fast as *N. ponderosa*, attaining a mean height of 17 mm in about a year. The acetate peel technique was used to age clam shells and confirmed that ages of average market-sized ponderous arks ranged from 6–13 years old. A growth curve ( $Y = 11.38 + 17.38 [\log X]$ ) derived from shell aging data indicated a growth rate of about 1 mm/yr after age 13 for *N. ponderosa*. Fisheries samples revealed that *N. ponderosa* constitutes the majority of clams being harvested in the blood clam fishery (mean height = 56 mm). The size-frequency distributions of non-fisheries samples (mean height = 40 mm) suggested that some areas were being overfished.

**POPULATION FLUCTUATIONS, DENSITY DEPENDENCE AND STOCHASTIC VARIATION IN THE BLUE CRAB.** Jacques van Montfrans\*, Romuald N. Lipcius, Karen S. Metcalf and Robert J. Orth, Virginia Institute of Marine Science, School of Marine Science, The College of William and Mary, Gloucester Point, VA.

The blue crab, *Callinectes sapidus*, is a dominant and ecologically important predator which is commercially harvested throughout its range. In Chesapeake Bay, blue crab population abundance fluctuates widely between years. We describe a comprehensive approach to the analysis of population variation in this species based on a stage-specific analysis of regulatory and stochastic processes driving survival and abundance of the various life-history stages (larva through adult). Laboratory and field experiments examined habitat influences upon survival of crabs <15 mm in carapace width and indicated that differential mortality between habitats, active habitat selection by postlarvae, and migration of the youngest juveniles jointly explain the non-random distribution of crabs in the field. We postulate that stochastic factors are primary important in the larval and postlarval stages, whereas habitat-related, density-dependent processes such as predation and cannibalism and migration regulate the juvenile phase. Further analysis based on collective field and laboratory experiments and long-term field sampling of postlarvae, juveniles and adults in Chesapeake Bay provide additional support for this perspective. These patterns and processes are related to recruitment relationships, habitat utilization and population dynamics in the blue crab.

**SPATIAL VARIATION IN GROWTH OF RAFT-CULTURED BLUE MUSSELS, *MYTILUS TROSSULUS*, IN PUGET SOUND, WASHINGTON (USA) ON THE NORTH-EASTERN PACIFIC COAST.** Karl W. Mueller, West Coast Blue Mussel Company, P.O. Box 207, Coupeville, WA 98239.

Recent studies in Spain, a leading producer of raft-cultured mussels, have indicated within-raft differences in growth rates of Mediterranean mussels, *Mytilus galloprovincialis*. Practical raft management is affected by such variation in growth. Given the lack of similar studies in the northeastern Pacific, I quantified the effects of raft component (east and west), culture rope position (outside, middle, and inside), and depth (1, 3, and 5 m) on specific growth rates (expressed as % shell length per day) in raft-cultured blue mussels, *Mytilus trossulus*, in northern Puget Sound during the fall of 1993.

Specific growth rates differed by as much as 28% after the 90-day experimental period. Variation in growth was detected along and across the raft, and with depth on the culture ropes. A 3-way ANOVA revealed 2 first-order interaction effects, raft component  $\times$  depth and culture rope position  $\times$  depth. The first interaction effect was due to the significantly higher growth rate of mussels at 3 m vs. 5 m on the east component of the raft, and the significantly higher growth rate of mussels located on the east component of the raft at 3 m compared to those grown on the west component at the same depth. The second interaction effect was due to significantly higher growth rates of mussels at 1 and 3 m compared to 5 m on outside culture ropes, and the significantly higher growth rate of mussels on outside culture ropes at 3 m vs. those at the same depth on culture ropes suspended in the middle of the raft.

I attributed variation in growth of raft-cultured *M. trossulus* to environmental factors and culture methods. Commercial farmers may maximize available food to mussels and optimize growth by rotating rafts on their axes part-way through the growing cycle, adjusting the number, position, and length of culture ropes suspended from rafts, and by removing fouling organisms from anti-predator devices such as nets.

**THE PACIFIC COAST OYSTER INDUSTRY—FACTORS CONTRIBUTING TO ITS LONGEVITY AND SUSTAINABILITY.** Terry Y. Nosh, Marine Advisory Services, Washington Sea Grant Program, University of Washington, Seattle, WA 98195.

Pacific oyster, *Crassostrea gigas*, farming is a sustainable process requiring the planting of seed, growth to maturity, processing and marketing and so is similar to the cultivation of other farmed products like corn or wheat. Oyster farming is a light industry that is highly compatible with the environment and produces virtually no pollution. Energy requirements are minimal with highest energy usage in the hatchery and juvenile nursery phases. Various algal cultures are used to feed larval and early juvenile oysters, but

most of the growth occurs as the oyster feeds from natural sources. Thus, feed costs are minimal.

The industry essentially began in 1922 when the first shipment of oyster seed arrived from Japan to the Rock Point Oyster Co. in Washington. All subsequent shipments were made from the same area in Japan, Miyagi Prefecture. Prior to this introduction, Olympia oysters (*Ostrea lurida*) were farmed extensively in Washington. Only small amounts of Olympia oysters are farmed today. At the time *C. gigas* was introduced, no other *Crassostrea* species existed on the Pacific coast except for the imported eastern oyster *C. virginica* which never did well on commercial beds. Because *Crassostrea* species on the west coast are lacking, the potential for disrupting gene pools of natural populations is non-existent.

Currently, this shellfish industry is one of the most sophisticated in the world. Key elements contributing to sustainability are tideland ownership, water quality, technology and innovation, marketing, and entrepreneurship. Principal concerns within the industry include: potential declines in growing area water quality, the issue of burrowing shrimp control, increasing regulations and user fees, and the media.

**THE USE OF WAX TO CONTROL THE SHELL PARASITES OF RED ABALONE, *HALIOTIS RUFESCENS*.** Frank R. Oakes, Raymond C. Fields, Peter F. Arthur and George A. Trevelyan\*, The Abalone Farm, Inc., P.O. Box 136, Cayucos, CA 93430.

Shell parasites can have a dramatic effect on the profitability of abalone culture. One such parasite, a recently discovered sabellid polychaete, interferes with abalone shell growth, resulting in a stunted, deformed shell lacking respiratory pores. The objective of this work was to develop a control method for this pest. Forty potential control agents were screened. A molten wax dip was selected as the most promising treatment. Various wax formulations, temperatures, and handling methods were compared in a series of 16 trials using over 1000 infected abalone (10–30 g). Percent sabellid kill and abalone survival were measured 3–8 days after the treatment. One treatment was found that repeatedly killed 91–99% of the sabellids. 6–18% of the abalone also suffered mortality as a result of the treatment. This treatment, which consisted of a 1–3 second dip in a 44°C wax mixture, produced a wax coat over the outer shell surface. After 3–6 days, this coat caused anoxia within the sabellid burrows, resulting in the death of larval, juvenile and adult sabellids. A group of 75 wax dipped abalone was grown out for 3 months alongside an untreated control group. Only 1% of the control abalone completed a new respiratory pore during this period, while 43% of the treated abalone did so. Mean final shell length of the treated abalone was significantly greater than that of the control abalone. Thus, molten wax dipping appears to be an effective and environmentally benign tool for controlling a particularly troublesome parasite of abalone. More work is needed to streamline this procedure.

**GAMETOGENIC DEVELOPMENT OF JUVENILE OYSTERS AND THEIR CONTRIBUTION TO THE PROLONGED SPAWNING SEASON IN GEORGIA'S COASTAL WATERS.** Francis X. O'Beirn and Randal L. Walker, University of Georgia, Shellfish Research Lab., 20 Ocean Science Circle, Savannah, GA 31411–1011, Peter B. Heffernan, Marine Institute, 80 Harcourt Street, Dublin 2, Ireland.

This study was initiated to determine the contribution of "young of the year" oysters to a late season spawn and corresponding setting of spat late in the season in coastal Georgia (April–October). Collectors deployed in May 1993 had oysters by July in sufficient numbers and sizes ( $21.3 \pm 0.9$  SE mm) to enable histological analysis to be carried out. In July, oysters on the collectors were marked and 30 animals were sampled on a monthly basis, for 7 months. Gonadal indices (GI) corresponding to: Ripe = 5, Late Active = 4, Early Active = 3, Partially Spent = 2, Spent = 1, and Inactive = 0, were used. In July, the mean GI was  $4.7 \pm 0.11$  SE, indicating that many of the oysters, which were no more than 3 months old, had attained sexually maturity. The GI's through August and September were  $4.8 \pm 0.15$  SE and  $4.3 \pm 0.24$  SE, respectively, thus indicating some spawning by September. The mean GI in October was  $2.9 \pm 0.29$  SE, demonstrating a major spawn in this month. The mean GI was  $2.9 \pm 0.1$  SE by January, when the study was terminated. Five month old oysters had sexually matured and spawned, and thus contributed to the late season spawn and subsequent set. The mean size at sexual maturity was  $27.5 \pm 0.52$  SE mm. Sex ratios for the juvenile oysters over the course of the study did not differ significantly from a 1:1 ratio ( $p = 0.332$ ), using a  $\chi^2$  test.

**ACUTE OSMOTIC TOLERANCE OF CULTURED CELLS OF THE OYSTER PATHOGEN *PERKINSUS MARINUS* ACCLIMATED TO LOW SALINITY.** Caroline L. O'Farrell\*, Jerome F. LaPeyre and Eugene M. Burreson, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The acute effect of salinity changes on cultured *Perkinsus marinus* meronts was examined to determine the osmotic tolerance of cultures that have been acclimated to low osmolality growth media. Culture *P. marinus* meronts acclimated for 5 months in media equivalent to 3, 6, 9, 12 and 22 ppt at a temperature of 27–28 C were incubated in buffered artificial seawater (ASW) treatments prepared at 0, 3, 6, 9, 12 and 22 ppt. Following a 24 hour incubation at 28 C, cell mortality was determined by uptake of the vital stain neutral red. Percent mortality of meronts acclimated to low salinity media was lower than that of meronts acclimated to higher salinity media when exposed to the low salinity ASW treatments. For example, in the 0 ppt ASW treatment, mor-

tality of meronts acclimated to 3 and 6 ppt media was 36 and 70%, respectively, whereas mortality of meronts acclimated to each of 9, 12 and 22 ppt media was 100%. In addition, meronts acclimated to low salinities survived the high salinity treatments as well as meronts acclimated to higher salinities. For example, in the 22 ppt ASW treatment, mortality was less than 4% for all acclimated cultures. These results suggest that *P. marinus* meronts acclimated to low salinity survive even in fresh water for at least 24 hours and that *P. marinus* is more tolerant of hyperosmotic stress than hypoosmotic stress.

**EFFECTS OF TRIBUTYL TIN EXPOSURE ON OYSTER (*CRASSOSTREA VIRGINICA*) DEFENSE FUNCTIONS.**

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Adult oysters (*Crassostrea virginica*) were exposed to 0, 0.03 or 0.08 ppb tributyltin oxide (TBT), an antifouling agent that leaches into the marine environment from treated boat hulls. Oysters ( $n = 12$ ) were evaluated for hemocytic defense functions and serum elements after 0, 2, 4 and 8 weeks of exposure, to investigate whether TBT exposure would cause changes in defense capacity. In a previous study (Fisher et al. 1990), exposure to 0.4–400 ppb TBT *in vitro* inhibited both hemocyte rate of locomotion and hemocyte chemiluminescence (CL), an indicator of reactive oxygen intermediate (ROI) production. After 2 weeks of *in vivo* TBT exposure, both the rate of hemocyte locomotion and the percentage mobile hemocytes were reduced in TBT-exposed groups as compared with the control group. At 4 and 8 weeks, no differences in hemocyte locomotion were seen between treatment groups. The average serum protein level in the 0.08-ppb group was significantly elevated ( $p < 0.02$ ) compared to 0 and 0.03-ppb groups after 2 weeks exposure, but at 4 and 8 weeks a dose-dependent reduction in protein was found. Serum lysozyme was significantly ( $p < 0.08$ ) lower for the 0.08 group compared to controls after 8 weeks. Whereas hemocyte locomotion and serum factors may have been suppressed by TBT exposure, CL measurements showed variable results. At all sampling times, unchallenged CL appeared to increase with increasing TBT concentration (0.08-ppb significantly higher than controls,  $p < 0.002$ , at 2 and 4 weeks). CL produced by hemocytes challenged with zymosan particles appeared to be inhibited at the 8 week sampling period. It is possible that increased "resting" levels of ROI production apparently induced in this experiment by TBT exposure reflected sublethal stress, while the hemocyte CL response when stimulated by zymosan particle addition was relatively unaffected.

**SENSORY ANALYSIS OF THREE GIANT CLAM SPECIES: *TRIDACNA GIGAS*, *TRIDACNA DERASA*, AND *HIPPOPUS HIPPOPUS*.** Stephanie Peavey\* and John Riley, University of Maine, Orono, ME 04469 (both formerly with the National Aquaculture Center, Federated States of Micronesia).

Three major species of giant clams grown in the Pacific region are *Tridacna gigas*, *Tridacna derasa*, and *Hippopus hippopus*. Presently, foodservice represents the biggest marketing potential, especially in strong tourist markets. Among restaurants catering to tourists, sashimi is the most popular preparation for giant clams. This research was initiated to determine if consumers could detect differences in the sensory characteristics of these three major giant clam species and if so, which species were most appropriate for marketing as sashimi fare. A sensory test was designed to evaluate how panelists rated the species in four sensory categories: 1) appearance; 2) texture; 3) taste and; 4) overall appeal. The results of the statistical analysis of ratings given by 42 panelist, showed that *T. derasa* consistently received the highest ratings overall and should be the featured species in establishing giant clams in the foodservice market. *H. hippopus* received sufficiently high ratings to warrant further consideration for foodservice promotion. *T. gigas*, however, generally received the lowest ratings overall. In comparison to the other species, *T. gigas* should not be promoted for use as sashimi in foodservice but may be appropriate for other preparations.

**GENETICALLY MODIFIED FINFISH AND SHELLFISH: PROSPECTS FOR THE 21ST CENTURY.** Dennis A. Powers, Hopkins Marine Station, Stanford University, Pacific Grove, CA.

During the last 2 decades, the application of recombinant DNA and hybridoma techniques to fundamental and practical problems in the biomedical and agricultural sciences has resulted in stunning victories, and the successful transfer of some of that information into the commercial sector has been equally impressive. Public awareness of the triumphs surrounding biotechnology has generally revolved around high profile advances in the diagnosis and treatment of diseases; however, equally important has been the production of fine chemicals, including pharmaceuticals and antibiotics. In addition to accomplishments in the biomedical field, there have been astonishing achievements in agriculture, including the generation of transgenic animals and plants, the creation of recombinant vaccines for the treatment of animal and plant diseases, and the manipulation of microbes to improve everything from soil nutrients to protection of plants against frost.

The oceans cover more than 70 percent of the earth's surface and fresh water ecosystems contribute a significant portion of the

remaining 30%. Because of the great depths of the oceans and other aquatic environments, these ecosystems compose over 95% of the world's biosphere by volume. The depths of these fresh and salt water habitats contain a multitude of diverse organisms. This tremendous biodiversity is essentially an infinite reservoir of model organisms for fundamental biomedical research and a tremendous resource for high quality food, biomedically important substances, sources for the biodegradation of anthropogenic waste and industrial pollutants, antibiofouling and anticorrosion substances, biosensors, biocatalysts, biopolymers, and other industrially important compounds and fine chemicals.

Even though the application of biotechnological efforts toward aquatic environments has been modest when compared to those efforts devoted to terrestrial systems, there have already been some notable successes and preliminary evidence that the entire field of marine biotechnology is becoming a new frontier of explosive scientific discovery with exciting global economic implications for the twenty first century. This paper provides selected examples of exciting scientific achievements within the field of marine biotechnology and points toward newly emerging areas that have a high probability of scientific and economic success within the next decade. Particular emphasis will be paid to: 1) the molecular cloning and expression of specific genes to produce reagents and vaccines for aquaculture, and 2) the generation of fast growing, disease resistant transgenic finfish and shellfish for aquaculture. Examples will be cited for genetic engineering of fast growing fresh and salt water finfish (e.g., carp, catfish, trout, tilapia, salmon, etc.) and shellfish (e.g., oyster and abalone) species. Considerations for environmental and economic safety will be presented as well as the need to educate the public concerning the fundamental science behind genetic enhancement and genetic engineering.

**THE ROLE OF BIVALVES AS FOULING ORGANISMS OF EUROPEAN FLAT OYSTERS, *OSTREA EDULIS* L., GROWN IN HANGING CULTURE.** Norman L. C. Ragg, Corrientes 2988-3°F, 1193 Buenos Aires, Argentina.

Reports of heavy fouling biomass accumulations within lantern nets used for the on-growing of Flat Oysters (*Ostrea edulis* L.) by the VIDESA long-line culture company, Algarve, Portugal, prompted an investigation into the extent and nature of the fouling at this site. Clean *O. edulis* valves were presented as a substratum within lantern nets to examine monthly fouling settlement over a 5 month experimental period (March to August 1993) and nets containing live oysters were correspondingly sampled at monthly periods. 83-95% of the fouling dry weight was found to be attrib-

utable to bivalve mollusca and the study, therefore, concentrated on the factors responsible for the extent to bivalve fouling and the effects of the presence of these mollusca. Each bivalve species was considered in terms of biomass and of the surface area presented as substrata for further fouling; the Mediterranean Mussel, *Mytilus galloprovincialis*, was found to dominate fouling biomass and the effects of this species' byssus were also considered, as they bound the mussels and oysters into a stable mass, aggravation the effects of crowding. Experimentation using *Mytilus edulis* in the Menai Strait (N. Wales) showed filamentous epifaunal fouling density to be a function of shell size, on live bivalves, but random on dead shells. Larval bivalve settlement density was found, in the absence of other variables, to be directly correlated to the amount of filamentous surface fouling and, consequently, clean dead valve immersed for one month periods were not considered to be suitably standardized to represent comparable monthly bivalve settlement trends on the oysters in culture. It was concluded that the major factors contributing to the extent of bivalve fouling were the presence of large amounts of filamentous epifauna on the oysters, stimulating settlement, and the lantern netting, and associated fouling, retaining unattached juvenile mollusks. The most important effects of fouling bivalves in this situation are suggested to be in terms of increased biomass and as spatial and trophic competitors to the oysters. Suggestions are made for further research and measures to reduce bivalve fouling. This work was carried out using the facilities of the School of Ocean Sciences, Menai Bridge, University College of North Wales (U.K.).

**CHANGES IN THE DENSITY AND SURVIVAL OF NEWLY SETTLED BIVALVE POST-LARVAE IN WILLAPA BAY, WA DUE TO THE INVASION OF SMOOTH CORDGRASS, *SPARTINA ALTERNIFLORA* LOISEL.** Stephen-Ratchford\* and Gilbert Pauley, Washington Cooperative Fish and Wildlife Research Unit, WH-10, University of Washington, Seattle, WA 98105.

*Spartina alterniflora* is an exotic species of cordgrass which is quickly changing the hydrology, geomorphology, and biology of Northwest estuaries such as Willapa Bay. We hypothesized that bivalves which inhabit the upper intertidal may be affected by *Spartina* during and after settlement. During summer 1993 we compared the density and survival of newly settled post-larvae (soft-shell clam, *Mya arenaria*) within and near live *Spartina* clones with that of the adjacent mud flats in Willapa Bay. Clam density at settlement was found to be highest along the inside perimeter of the patches. At 4 months post-settlement, clam density was highest on the mud flat. A second field season was conducted in summer 1994 to investigate these patterns further.

**EFFECTS OF FLOW RATE AND STOCKING DENSITY ON GROWTH AND SURVIVAL OF OYSTERS (*CRASSOSTREA VIRGINICA*) AFFECTED BY JUVENILE OYSTER DISEASE IN AN UPFLOW NURSERY SYSTEM.** Gregg Rivara\*, Cornell Cooperative Extension—Suffolk County, 39 Sound Avenue, Riverhead, NY 11901, Stanley Czyzyk, Blue Points Company, P.O. Box 8, West Sayville, NY 11796.

Juvenile oyster disease (JOD) first affected the east coast (USA) oyster industry in 1988. Losses of close to 100% have occurred in seed less than 25 mm shell height. This experiment was designed to confirm earlier work that showed relatively high flow rates and low stocking densities in upweller nursery silos reduced mortalities related to this disease. A 3-tank, 27-silo upflow nursery system was constructed. Each tank served as a flow treatment: low (4 Lpm per silo), medium (20 Lpm) and high (40 Lpm). Within each tank 3 stocking densities were triplicated: low (1 L per silo), medium (6 L) and high (12 L). Silo tanks were fed unfiltered creek water from a 3.4 m<sup>-3</sup> head tank filled by two 2 hp pool pumps. Silo units were initially stocked on 11 July 1994. Shell height at this time averaged 8 mm. Each week, for a period of 9 weeks, the volume and mortality (dead/100 animals) of each silo, along with shell height measurements (live and dead) were recorded. Physical parameters measured included temperature, salinity, secchi depth and a check of flow rates. As the actual volume exceeded the target volume for each silo, oysters were randomly removed each week to bring the volume back down to the treatment level. Substantial mortalities (>20%) were seen by week 3 in low flow silos; by week 4 mortalities in low flow silos were 50–70%. At this time high flow silos were at or under 33% dead, and had not changed substantially by the end of the trials. Weekly volumetric increases were significantly higher in high and medium flow silos as compared to low flow silos. Flow affected growth and survival more than stocking density. It is not known how much nutritional stress affects the manifestation of JOD. It is suggested that silos can be highly stocked with relatively high flow rates (0.03 L min<sup>-1</sup> cm<sup>-2</sup> silo screen area) in order to reduce mortalities of oysters exposed to JOD.

**THE SUMINOE OYSTER: READY FOR COMMERCIAL PRODUCTION.** Anja M. Robinson\* and C. J. Langdon, Department of Fisheries and Wildlife, Oregon State University, Hatfield Marine Science Center, Newport, OR 97365.

The Suminoegaki oyster *Crassostrea ariakensis* (Fujita 1913) was introduced to the west coast of the United States from Japan. It does not reproduce in cool waters and becomes sexually mature in late summer. Sexual maturation can be accelerated by holding adult oysters at elevated water temperatures (20°C). Conditioned broodstock oysters can be spawned by temperature shock. Larvae were raised to setting and metamorphosis by culturing at 25°C in 20 ppt seawater, fed on a mixed diet of the flagellate *Pseudoisochrysis paradoxa* and the diatom *Chaetoceros calcitrans*. Using the techniques developed at the Hatfield Marine Science Center in

Newport, Oregon, commercial hatcheries have been able to raise larvae through setting and metamorphosis.

Cultchless spat was obtained by setting eyed larvae on shell chips. Cultchless spat was held in upwellers until large enough to plant at growout sites. Growth of planted spat varies at different locations.

#### **PACIFIC LITTLENECK CLAM, *PROTOTHACA STAMINEA* INCEPTIVE FARMING PLAN FOR ALASKA.**

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Recently there has been much interest in culturing native Pacific littleneck clams in Alaska. This is the result of several factors. Markets are strong for littleneck clams and many Alaskan oyster farmers want to diversify production on rented state land. There are numerous beaches in southeast and south-central Alaska supporting large populations of littleneck clams, but wild fishery harvests are limited to beaches in certified shellfish growing areas, which represent a small percentage of suitable clam habitat in Alaska. Shellfish import regulations prohibit the introduction of the more commonly cultured Manila clam, *Venerupis japonica*. Encouraged by the success of Manila clam farming in the Puget Sound area of Washington, Alaska's growers hope that cultivation of Pacific littleneck clams will increase yield and shorten the interval between harvests. A small-scale (half acre) commercial clam farming permit was conditionally approved this year (1994). That approval establishes a regulatory precedent allowing commercial clam cultivation on public beaches (tidelands in Alaska are state owned). Seed sources are currently limited to wild stocks. Initial standing crop estimates indicated high natural recruitment levels; therefore, annual area seeding is anticipated. Bivalve competitor culling and thinning of littleneck clam standing crops is proposed. A degree of protection from predators will be achieved by covering culture plots with plastic mesh netting. Whole area harvesting/culling is proposed every other year. Based on a seven year growth cycle, the projected annual yield is 86 to 108 marketable clams per m<sup>2</sup>.

#### **INDUCTION OF TRIPLOIDY IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, USING 6-DMAP.**

**John Scarpa\***, **David E. Vaughan** and **Ross Longley**, Harbor Branch Oceanographic Institution, Inc., 5600 U.S. 1 North, Ft. Pierce, FL 34951.

Cytochalasin B (CB) is the most widely used chemical for inducing triploidy in bivalves and is the benchmark for comparing other potential inducers. Cytochalasin B has Food and Drug Administration approval but this may be reviewed. An alternative chemical inducer, 6-dimethylaminopurine (6-DMAP), that is water soluble and shown to be an effective inducer of triploidy in the Pacific oyster, giant sea scallop and blue mussel (Desrosiers et al. 1993. *J. Exp. Mar. Biol. Ecol.* 170:29-43) was tested on the

eastern oyster *Crassostrea virginica*. Various 6-DMAP concentrations were tested and compared to CB (0.3 mg/L) and a non-treated control. Eggs and sperm were obtained by dissection and kept at 25°C and 30 ppt sea water. Fertilized eggs were divided into treatment groups with exposures beginning 20 min and ending 35 min post-insemination. Larvae were cultured for 48 hr and then sampled for development and ploidy analysis. In experiment I the 6-DMAP concentrations tested were 200, 400, 600 and 800 µM. Flow-cytometric analysis revealed 100% diploidy in non-treated, 17% diploidy and 83% triploidy in CB, and 85% diploidy and 15% triploidy in 200 µM 6-DMAP. Concentrations above 200 µM produced poor readings. In experiment II the 6-DMAP concentrations tested were 50, 100, 200 and 400 µM. Flow-cytometric analysis revealed 100% diploidy in non-treated, 100% triploidy in CB, and 100% diploidy in 50-200 µM 6-DMAP. Mean size of larvae decreased with increasing 6-DMAP concentration (50-78.5 µm; 100-75.3 µm; 200-74.3 µm; 400-71.0 µm) and were generally smaller than the non-treated (79.3 µm) and CB treated (76.7 µm). 6-DMAP concentrations of 200 µM and above reduced survival drastically (<20% compared to CB and non-treated). The advantage of 6-DMAP being water soluble, thereby reducing worker exposure to DMSO when using CB, may be offset by its low triploid induction efficiency and survival of larvae. Further investigation of exposure timing may increase the efficacy of 6-DMAP for use with the American oyster.

#### **OPTIMAL STOCKING DENSITY AND SUBSTRATE SIZE FOR THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, IN A HATCHERY SETTING SYSTEM.**

**Kimberly B. Simmons\***, **Valerie L. Shaffer** and **Mark W. Luckenbach**, The College of William and Mary, Virginia Institute of Marine Science (VIMS), School of Marine Science, Gloucester Point, VA 23062.

US Eastern oyster production is particularly significant in Chesapeake Bay area where over harvesting, diseases, and pollution have contributed to an alarming decrease in oyster production, from 32 million pounds in 1959 to less than one million in 1991. In response to declining oyster production the state of Virginia has a research hatchery to investigate the technical and economic feasibility of oyster seed production in Chesapeake Bay. Our objective was to optimize hatchery setting procedures for the mass production of *Crassostrea virginica* seed by minimizing multiple strikes, which would reduce marketability, while maximizing survival and growth. Separate experiments were conducted to determine optimal stocking density and substrate (crushed oyster shell) size for oyster pediveligers. In each experiment larvae from single cohorts produced under mass production conditions were added to downweller units (Bottom surface area = 500 cm<sup>2</sup>) coated with paraffin wax. Seven substrate treatments were tested with size operationally defined by retention on 0.3, 0.5, 1.0, 1.4, 2.0, and 4.75 mm sieve series, as well as a mixed range (1.0-2.0 mm) treatment. Two hundred and ten thousand pediveligers were placed into downweller units each of 3 replicates containing the

appropriate substrate and allowed to settle. Five stocking densities ranging from 50,000–150,000 pediveligers per downweller unit at intervals of 25,000 were tested using a single substrate size (0.5 mm). Maximum survival and minimal multiple strikes were observed on the 0.5 mm crushed shell. Survival and single strikes were highest in the 50,000 larvae/unit (100 larvae/cm<sup>2</sup>) and 100,000 (200 larvae/cm<sup>2</sup>) treatments. These findings, thus, suggest that a substrate size of >0.5 mm and <1.0 mm with stocking density of 100,000 provide for optimal setting conditions within this context.

**TEMPORAL CHANGES IN PHYTOPLANKTON IN A LOUISIANA ESTUARY: TROPHIC AND DYSTROPHIC EFFECTS ON THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.** Thomas M. Soniat\*, and Geoff A. Mire, Department of Biology, Nicholls State University, Thibodaux, LA 70310, Randy J. Robichaux, and Quay Dortch, Louisiana Universities Marine Consortium, 8124 Highway 56, Chauvin, LA 70344.

Phytoplankton populations over oyster reefs in the Terrebonne Basin of Louisiana were sampled weekly through an annual cycle. Identification of phytoplankton has provided important qualitative information on possible available food for the eastern oyster and has revealed that potentially toxic species are present in the low salinity (0.5–20.0 ppt), high turbidity (8.0–244.0 mg/L SPM) estuaries of Louisiana. Previous studies have shown that chlorophyll *a* probably underestimates the food available to oysters. Small cyanobacteria (<3 μm), which have a low chlorophyll content, may account for the discrepancy because they are the most abundant phytoplankton in the summer and are commonly found in the oyster digestive system. Despite their small size, they may play an important role in the summertime nutrition of oysters in turbid, subtropical estuaries if they are included in larger sediment aggregates. Phytoplankton which produce human toxins which accumulate in shellfish, including *Pseudonitzschia* spp., *Dinophysis* spp., and *Prorocentrum* spp., and phytoplankton which may produce fish and oyster toxins, such as *Heterosigma* sp. and *Gymnodinium splendens*, have been observed, sometimes at concentrations exceeding 10<sup>5</sup> cells/L. The presence of toxic species in the absence of any documented effect on Louisiana oysters or consumers raises the question: Are these species an incipient threat to the Louisiana oyster industry?

**IMPLICATIONS OF ALTERNATIVE ECOLOGICAL STRATEGIES ON THE DYNAMICS OF BERING SEA CRAB POPULATIONS.** Bradley G. Stevens, NMFS, P.O. Box 1638, Kodiak, AK 99615.

Collective landings from 6 different species of Bering sea crabs constitute the most productive crab fisheries in the world. All 6 species exhibit extreme abundance fluctuations and sporadic recruitment. However, while 3 stocks of king crabs (Anomura: Lithodidae) have exhibited low recruitment for over 20 years, Tanner and snow crabs (Brachyura: Majidae; *Chionoecetes* sp.) have experienced 2 major recruitment events during that time period. Differences in recruitment patterns for these 2 groups of

crabs may be partially explained by differences in life history strategies. Such differences include location and concentration of spawners, sensitivity to climatic fluctuations, distribution of critical juvenile habitats, susceptibility to predation, aggregative behavior, and incidental mortality due to fishery discards and ghost fishing. Each of these ecological aspects is compared between the two groups for its potential impacts on recruitment and population dynamics.

**INBREEDING STUDIES ON THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*.** Sheila Stiles and Joseph Choromanski\*, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, 212 Rogers Avenue, Milford, CT 06460.

Inbred lines are being developed for the bay scallop, *Argopecten irradians*, to measure effects of inbreeding, as well as to exploit the potential for producing homozygous scallops with outstanding characteristics, such as fast growth or increased muscle yield. Inbreds could be selected within lines or families for particular traits, then crossbred for heterosis. Inbred scallops also can be developed to evaluate environmental conditions, as they are more genetically uniform, and therefore should demonstrate a more striking response to external changes. For the present study, scallops were selected based on shell color or depth to use for inbreeding by self-fertilization. For example, the parent scallop for one inbred line had a striking yellowish orange shell; most of its progeny had shells that were also yellowish orange. This line seems to be quite robust for growth and survival. Another scallop that was self-fertilized had shells with 35 broad white stripes and the majority of its progeny had stripes that ranged from 1 to 5 with varying widths. This line appears to have somewhat lower viability. Generally, in contrast to mass-spawned cultures with survival to metamorphosis in 4 out of 4 crosses, only 4 of 8 self-fertilized cultures had larvae that survived to metamorphosis. In addition, development to 48 hours was significantly lower, and growth was retarded in the inbred scallop cultures, suggesting inbreeding depression. Preliminary results indicate the following: 1) scallops manifest inbreeding depression in early stages by decreased survival to the larval stage and to metamorphosis, and by retarded growth of larvae and early juveniles; 2) shell marks seem to be reflective of genotypes with a significant genetic component; 3) different inbred lines will have different degrees of fitness. The bay scallop, a functional hermaphrodite, provides an interesting model for genetic studies of bivalves to rapidly develop inbred lines through self-fertilization.

#### A SENSITIVE AND SPECIFIC DNA PROBE FOR THE OYSTER PATHOGEN *HAPLOSPORIDIUM NELSONI*.

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*Haplosporidium nelsoni* is a significant pathogen of the eastern oyster, *Crassostrea virginica*, along the middle Atlantic coast of the U.S. To prepare a DNA probe for use in life cycle studies, genomic DNA was extracted from *H. nelsoni* plasmodia and the small subunit (SSU) rDNA was amplified by PCR, cloned and sequenced. The sequence of *H. nelsoni* rDNA was aligned with that of *Minchinia teredinis* and SSU rDNA data of oyster and various protists in GenBank. A unique 20-base oligonucleotide labeled with digoxigenin was commercially synthesized and tested for sensitivity and specificity. In dot-blot hybridizations the probe readily detected 10 pg of cloned *H. nelsoni* rDNA or the presence of *H. nelsoni* in only 1 µg of genomic DNA from infected oyster tissue, and did not hybridize with cloned SSU rDNA of *C. virginica*, *Perkinsus marinus* or *M. teredinis*. The probe was further tested for specificity with *in situ* hybridizations on alcohol/Formalin/acetic acid-fixed, paraffin-embedded tissue sections. The probe hybridized well with *H. nelsoni* plasmodia and immature spores, but poorly with mature spores. The probe did not hybridize with oyster tissue, with other common oyster parasites such as *P. marinus* or *Nematopsis* sp., or with the haplosporidians *Haplosporidium louisiana* from mud crabs (*Panopeus* spp.), *Haplosporidium costale* from *C. virginica* or *M. teredinis* from shipworms (*Teredo* spp.).

**DEVELOPMENT OF A SHELLFISH AQUACULTURE INDUSTRY ON THE WEST COAST OF FLORIDA: SEED PRODUCTION, GROWOUT MONITORING, AND SPECIES DIVERSIFICATION.** Leslie N. Sturmer\*, and Everette Quesenberry, Harbor Branch Oceanographic Institution, Inc., Cedar Key, FL 32625, John Scarpa and David E. Vaughan, Aquaculture Division, Ft. Pierce, FL 34946. Private.

A shellfish aquaculture industry has recently emerged on the west coast of Florida as a result of a JTPA-funded retraining program, Project OCEAN. Over 130 underemployed seafood workers were placed onto submerged land leases in 1993. In recognition of the support required by these new farmers, Harbor Branch Oceanographic Institution has maintained a land-based and field nursery in Cedar Key to provide shellfish seed as well as extension services. To date, about 15 million hard clam, *Mercenaria mercenaria*, seed have been planted, with sales expected to exceed 20 million seed in 1994. The commercial production of juvenile clams to nursery planting size (6 mm) and final planting size (12-15 mm) has allowed for comparison of upweller, raceway, and field systems. Further, seasonal variations were determined for field nursery plants in which 6-mm seed were stocked at 10,000/m<sup>2</sup> in 1.5 m<sup>2</sup> bottom bags. Survival of bottom plants ( $n = 9$ ) ranged from 58 to 88% over 50 to 75-day nursery periods.

Greatest growth was observed for June-planted seed (0.14 mm/day); whereas negligible growth (0.08 mm/day) occurred in clams planted in January. Growout production of hard clams was monitored by planting 12,000 to 16,000 14-mm seed in polyester bottom bags at rates of 660/m<sup>2</sup> during the winter, spring, summer, and fall of 1994. After 6 months, growth rates of 3.25 mm/month and 4.45 mm/month for the winter and spring plants, respectively, indicated a 12-month culture period for littleneck (50 mm) clams. Additionally, a commercial-scale evaluation of triploidy on performance of American oysters, *Crassostrea virginica*, in subtropical conditions was initiated in July (1994) by distributing over 300,000 triploids to 30 growers. Preliminary oyster production data will be presented. The culture potential of the bay scallop, *Argopecten irradians*, was also investigated by deploying field-collected scallops in rack-supported polyethylene bags at existing lease areas. Field observations were used to assess the future of this species for diversification of crops on shellfish aquaculture leases in Florida.

#### SPAWNING AND SPAT COLLECTION OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*, IN THE WESTPORT RIVER ESTUARY, MASSACHUSETTS.

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In response to poor recruitment of the bay scallop, *Argopecten irradians*, in the Westport River Estuary, MA, the Bay Scallop Restoration Project was initiated in 1993. The main objective of the project is to enhance scallop stocks by using spanner rafts and artificial spat collectors. Preliminary research of artificial spat collectors placed during summer 1993 at 11 locations within the Westport River indicated that Cowries Island was the most productive site, yielding 1,882 scallops. Research conducted during summer 1994 focused on this site to assess larval abundance and recruitment to artificial collectors. In December 1993, adult scallops were placed at Coreys Island and monitored bimonthly to assess gonadal maturation. From June to August 1994, artificial spat collectors (2 mm to 4 mm plastic mesh bags containing monofilament) were suspended on 20 to 30 ft floating longlines. A single longline, containing 25 bags, was deployed weekly for a period of 10 weeks. After 4 weeks soaking time, 5 bags were harvested from each longline to assess recruitment to spat collectors. Simultaneously, larval monitoring was conducted within close proximity to longlines and adult scallops to evaluate larval abundance and general distribution. Assessment over a 10 week period determined that peak spawning and recruitment occurred during mid-July. The use of adult gonadal monitoring and larval sampling allows for the proper setting of artificial spat collectors to optimize the resource and estimate recruitment potential. Spat harvested from collectors may be utilized for grow-out purposes to maintain spawner rafts, or may be reseeded into the estuary to

enhance natural stocks. This study indicates that spat collection methods for *A. irradians* may be beneficial to future resource management practices in New England by helping to improve, stabilize and restore scallop stocks.

**ENHANCEMENT OF THE SCALLOP *PECTEN FUMATUS* IN TASMANIA—JAPANESE TECHNOLOGY TRANSFER DOWN UNDER.** John D. Thomson\*, Toshikazu Fujimoto, Haruo Moriya and Tohru Ikeda, HOTAC Maritimes Pty Ltd., GPO Box 1386, Hobart, Tasmania 7001, Australia.

Following the collapse of the Tasmanian scallop fishery, scallop husbandry fishing techniques were transferred from Aomori and Hokkaido in Japan to Tasmania during the Tasmanian Scallop Enhancement Project 1987–1993. Tasmanian oyster hatcheries were unable to supply enough scallop spat for reseeding trials. Targeted wild spat collection of *Pecten fumatus* was successfully undertaken in commercial quantities from 1989 onwards. Intermediate culture was found to be necessary before reseeding at a minimum size of 40 mm shell length. On 4 different reseeding beds, survival was inversely proportional to growth. Shifting sand smothered scallops in storms. Lantern cage culture produced marketable (80 mm shell length) scallops 18 months from spatfall. Ear hanging culture, a less capital intensive alternative provides a similar product and broodstock for the next generation of spat. If a high survival rate can be achieved for reseeding, reseeding is the most suitable method of scallop enhancement in Tasmania. If survival rates cannot be improved, then hanging culture represents a feasible enhancement alternative but at greater cost. From inception to conclusion the Tasmania Scallop Enhancement Project increased the number of spat caught by a factor of 305, the number of intermediate cultured juveniles by a factor of 250, the number of reseeding juvenile scallops by a factor of 4,500 and the number of hanging culture scallops by a factor of 25. The current annual production is 10 million scallops, some 2 million from reseeding and 8 million from hanging culture. The success to date should be sufficient to industrialize the Project completely.

**SPAT COLLECTION AND GROWTH OF THE ICELAND SCALLOP, *CHLAMYS ISLANDICA*, (O. F. MÜLLER) IN SUSPENDED CULTURE IN ICELANDIC WATERS.** Guðrún G. Thórarinsdóttir. Marine Research Institute, P.O. Box 1390, IS-121, Reykjavík, Iceland.

During July 1988–September 1991, a study was carried out to evaluate spat collection and the potential of pearl net cultivation of Iceland scallop, *Chlamys islandica* (O. F. Müller), in Breidafjörður, on the west coast of Iceland. Spat was artificially collected, using polyethylene bags of 4.5 mm mesh size filled with monofilament of 0.2 mm. The spat collectors were placed at 25–35 m depth in Breidafjörður in the middle of July 1988. The settlement of the spat was observed in the middle of September. The instantaneous growth rate was highest after the settlement in September to the middle of October (3.6%/day) but decreased during the

winter. In May 1989 the growth increased again concurring with an observed increase in sea temperature and abundance of phytoplankton. In September 1989 one year old spat (mean height 9.8 mm  $\pm$  2.0 s.d.) were transferred from collectors to pearl nets suspended from flotation at 6–8 m depth. Shell increment, chlorophyll-*a*, temperature and salinity were monitored each month at the experimental site. By September 1990 the scallops had an average shell height of 24.6 mm  $\pm$  6.0 s.d. and in September 1991, three years after settlement, a height of 43 mm  $\pm$  8 s.d. was reached. Growth rate was related to food availability (measured as chlorophyll-*a*). During both years of the pearl net culture height specific growth rate reached a maximum (0.7% day<sup>-1</sup> and 0.3% day<sup>-1</sup>, respectively) in early spring and again in summer, but it gradually decreased as the scallops grew older. The growth season lasted from March to October during both the years. The results showed that the growth of Iceland scallops can be increased markedly by suspending the animals in hanging culture in more favorable environmental conditions than found on the natural scallop beds.

**BIOCHEMICAL CHARACTERIZATION OF THE OYS-TER PARASITE, *PERKINSUS MARINUS*: LIPID AND FATTY ACID COMPOSITION.** Aswani K. Volety\*, Fu-Lin E. Chu and Sureyya Ozkizilcik, Virginia Institute of Marine Science, School of Marine Science, College of William & Mary, Gloucester Point, VA 23062.

The protistan parasite, *Perkinsus marinus* has caused severe mortalities in the eastern oyster, *Crassostrea virginica*. Both merozoite and prezoosporangia stages of *P. marinus* are characterized by an abundance of refractile bodies which are lipid droplets. To determine the role of lipids and fatty acids in the parasites' development, study is in progress to characterize the lipid and fatty acid composition of meront and prezoosporangia. Preliminary results indicate that merozoites contained significantly higher lipids than prezoosporangia ( $p < 0.05$ ). The lipid class composition of these 2 life stages was also different. Phospholipids (PL) were the major lipid class (61%) in cultured merozoites while triacylglycerols (TAG) were dominant in prezoosporangia (67%). The percentage of lipid classes composition in cultured merozoites was as follows: wax/cholesterol esters (WE/CE, 18%), TAG (18%), free fatty acids (FFA, 3%), PL (61%) whereas in prezoosporangia the lipid class composition was: WE/CE (10%), TAG (67%), FFA (1.7%), cholesterol (12.5%), and PL (8.3%). The dominance of PL in merozoites may be an indication of active assimilation of PL from the host for membrane synthesis. The lipid in merozoite cultured media was primarily WE/CE and PL. TAG are the usual form of storage lipids. Higher TAG in prezoosporangia may be an indication that TAG is the energy reserve for further development to zoosporangia stage. Our preliminary results also indicate that prezoosporangia has much higher level of arachidonic acid which is an essential fatty acid for mammals, (20:4w6, >12% of the total fatty acids) compared to the host, oyster (<2%). This suggests that

*P. marinus* may actively assimilate arachidonic acid and/or modify short chain fatty acids of w6 family from the host.

**EMBRYONIC AND LARVAL DEVELOPMENT OF THE SOUTHERN SURF CLAM, *SPISULA SOLIDISSIMA SIMILIS* (SAY, 1822).** Randal L. Walker, Francis X. O'Beirn\* and Dorset H. Hurley, Shellfish Research Laboratory, University of Georgia, Marine Extension Service, 20 Ocean Science Circle, Savannah, GA 31411-1011.

The embryonic and larval development of the southern surf clam is described. Eggs were  $58.5 \pm 0.32$  (SE) mm in diameter and range in size from 48.3 mm to 75.0 mm. The size frequency distribution of eggs was normal. For animals cultured at 25 ppt and 22-23°C, 50% of eggs exhibited germinal vesicle breakdown at 10 mins., first polar body formation at 22 mins., first division at 41 mins., second division at 89 mins., and third division at 115 mins. Animals attained blastula stage, gastrula stage, and early trochophore stage by 6 hrs, 8.7 hrs, and 15 hrs, respectively. Fifty percent of animals were D-stage by 16.8 hrs and pediveliger stages were observed by 7.5 days, when animals were 94 mm in shell length. No significant difference in egg size ( $\Delta O(x) = 58.3$  mm) occurred for animals conditioned for 4 weeks in 450 L tanks in the hatchery at 25 ppt and maintained at 15°C, 20°C, or 25°C. Significantly ( $p < 0.0001$ ) more larvae survived (48%) to 48 hrs from eggs produced by animals conditioned at 25°C; however, larvae ( $\Delta O(x) = 77.4$  mm) were significantly smaller after 48 hrs from animals conditioned at the other two temperatures ( $\Delta O(x) = 79.3$  mm for 15°C and  $\Delta O(x) = 79.4$  mm for 20°C). Larvae were cultured at 25 ppt at temperatures of 15, 20, and 25°C and at 20°C at salinities of 15, 25 and 30 ppt. Larvae grew best at 25 ppt and 20°C treatment with good growth at 30 ppt and 20°C. Poor growth occurred at the 15 and 25°C treatments with no growth or survival occurring in the low salinity (15 ppt) treatment.

**PROCESSES INFLUENCING TRANSPORT OF DECAPOD LARVAE THROUGH AN ESTUARINE INLET.** E. L. Wenner\*, C. A. Barans, D. M. Knott and B. W. Stender, Marine Resources Research Institute, Box 12559, Charleston, SC 29422, J. O. Blanton and J. A. Amft, Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411.

Inlets are critical areas for linking the offshore habitat of larvae with estuarine nursery areas. The physical dynamics associated with inlets may be a bottleneck to estuarine recruitment of decapod larvae, such as blue crab (*Callinectes sapidus*) and penaeid shrimp (*Penaeus* spp.), which are spawned offshore. Transport comprises 2 steps, each likely dominated by a different mechanism: 1) transport from seaward spawning or larval development sites into the coastal frontal zone, and 2) transport from the coastal frontal zone

into the estuary. A central hypothesis of an ongoing 4-year study is that postlarvae are entrained into an inlet by an interaction of the alongshore coastal current with a landward influx of coastal water caused by alongshore wind stress and tidal currents. Experiments utilizing a combination of moored instrumentation and shipboard surveys were conducted over the neap-spring cycle within and offshore of a South Carolina inlet. Two research vessels operated in the inlet and along transects offshore to measure currents, temperature, salinity and larval abundance. Upwelling and downwelling wind cycles affect the oceanography of coastal waters and may influence transport of larvae. Coupling of coastal and estuarine waters is optimized during periods when downwelling-favorable winds transport oceanic water into the estuary. Peak densities of postlarval *Penaeus* and megalopae of *Callinectes* are greatest near the surface and coincide with downwelling conditions. The vertical distribution of larvae affects the effectiveness of wind events in concentrating larvae at the coast.

**CONSUMER AWARENESS AND PERCEPTIONS OF FARM-RAISED SHELLFISH: IMPLICATIONS FOR MARKETING.** Cathy R. Wessells\*, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881, Bobby Gempesaw, Department of Food and Resource Economics, University of Delaware, Newark, DE 19717, Alberto Manalo, Department of Resource Economics, University of New Hampshire, Durham, NH 03824.

The demand for shellfish has been shown to be affected by consumers' concerns regarding the quality and safety of the product. In this study, we investigate the effect of 1) awareness of aquaculture production of shellfish, 2) familiarity with shellfish products, and 3) the level of confidence in the shellfish supply on consumer's perceptions of the quality of farm-raised product. The data used in the paper is drawn from a survey of consumers in the 13 northeastern and mid-Atlantic states plus the District of Columbia conducted in the summer of 1993. Of 5,000 surveys mailed to random households, 1,533 surveys were returned. The survey includes question related to consumer purchasing behavior for fresh shellfish products (mussels, oysters and clams) for consumption at home. Other questions include characterization of beliefs and attitudes regarding farmed versus wild shellfish. Many of these relate to product quality and safety aspects of farmed versus wild product. In addition, demographic information was collected. Equations are econometrically estimated which capture the influence of socioeconomic factors on consumer beliefs regarding the safety and quality of farm-raised shellfish in comparison to wild-harvested.

**ANALYSIS OF TOXIC ALGAE BLOOM EVENT ON CONSUMER DEMAND FOR MUSSELS IN MONTREAL.** Cathy R. Wessells, Priscilla M. Brooks and Chris Miller, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881.

Toxic algae blooms are a worldwide phenomena which appear to be increasing in frequency and severity. These natural events often have significant economic consequences on the aquaculture industry, including supply interruptions due to closed beds or stock mortality, and losses due to a decline in demand for the product. Past research on the economic impacts of harmful algal blooms focus on supply side losses (lost revenues), and primarily those incurred by wild fisheries. Another, mostly overlooked, economic impact of harmful algal blooms arise from media reports of the dangers of consuming affected seafood. Publicized reports that certain seafood are toxic create consumer fear and avoidance of those products. The result is a short term and possibly long term decline in demand for affected, and often for unaffected, seafood.

To investigate the potential size of the economic loss to the aquaculture industry, the proposed paper will look specifically at one event; a toxic bloom occurring in Prince Edward Island, Canada in 1987 which heavily impacted the cultured mussel industry in the Maritimes and Northeastern U.S. This incident caused 107 illnesses and three deaths from amnesic shellfish poisoning. The Canadian Health Minister issued a warning against consumption of all mussels, regardless of origin. Using firm level sales data (in cooperation with Great Eastern Mussel Farms of Tenants Harbor, Maine) we will estimate consumer demand for mussels in Montreal, a major mussel market, from 1986 through 1992. We will use proxy variables, following previous economic literature on demand effects of negative product information, to capture the effect of the event on consumer demand in both the short and longer term.

**DO DIFFERENCES IN DINOFLAGELLATE DAMAGE DEPEND UPON DIGESTION?** Gary H. Wikfors\*, NOAA, National Marine Fisheries Service, 212 Rogers Avenue, Milford, CT 06460, Roxanna M. Smolowitz, Laboratory For Marine Animal Health, University of Pennsylvania, Marine Biological Laboratory, Water Street, Woods Hole, MA 02543.

Differences in responses of bivalve mollusks to dinoflagellates have been reported often, but reasons for differences in susceptibility are poorly understood. Our recent studies have identified specific pathologies observed in two bivalve species exposed under controlled conditions to cultured strains of 2 dinoflagellates, *Prorocentrum minimum* and *Gymnodinium nelsoni*.

*P. minimum* was fed alone, and in combination with algae known to be good diets, to juvenile bay scallops, *Argopecten irradians*, and to several life-history stages of the eastern oyster, *Crassostrea virginica*. *P. minimum* was acutely toxic to scallops, causing complete mortality in 1–4 weeks accompanied by atrophy and necrosis of digestive gland absorptive cells and systemic effects (melanized hemocyte clots) characteristic of exposure to chemical toxins. Post-set oysters, by contrast, were not killed by

this dinoflagellate, but rather developed distinctive accumulation bodies within absorptive cells. Juvenile oysters then filtered, but did not consume *P. minimum* cells for approximately three weeks. Thereafter, oysters began consuming this dinoflagellate and growing well. A similar behavioral sequence of pseudofeces production for about 2 weeks followed by consumption and growth occurred when post-set oysters were exposed to *G. nelsoni*. Histologic changes in absorptive-cell appearance accompanied this behavioral sequence; accumulation bodies were no longer present in oyster absorptive cells after the 2–3 week refractory period. Contents of accumulation bodies in oyster absorptive cells appear to include undigested dinoflagellate autolysosomes, and other organelles, taken into oyster absorptive-cell food vacuoles during phagotrophy. Scallops are not phagotrophic, and thus digestion occurs entirely in the digestive lumen, where chemical toxins, including autolysosome enzymes, may be released from the dinoflagellate organelles and attack absorptive cells. Differences between oysters and scallops suggest that the site where the dinoflagellate organelles are digested—intracellular or extracellular, respectively—may play a role in determining response to a toxin.

**HIGH-LIPID TETRASELMIS CULTURES SUPPORT RAPID GROWTH OF POST-SET OYSTERS AND SCALLOPS.** Gary H. Wikfors\*, NOAA, National Marine Fisheries Service, 212 Rogers Avenue, Milford, CT 06460, Glenn W. Patterson, Department of Botany, University of Maryland, College Park, MD 20742, Ralph A. Lewin, University of California, Scripps Institution of Oceanography, La Jolla, CA 92093.

Our previous studies have demonstrated a positive correlation of growth of the eastern oyster, *Crassostrea virginica*, with contents of the essential fatty acids (20:5n3 and 22:6n3) and certain delta-5 sterols in diets of cultured phytoplankton. This correlation led us to screen marine microalgal strains for these biochemical compounds in an effort to identify promising nursery diets for bivalve shellfish. Marine algae isolated selectively for high lipid content were analyzed for fatty-acid and sterol contents, and several were found to contain appreciably more of the specific lipid compounds of interest; the best of these were from the prasinophyte genus, *Tetraselmis*.

We conducted feeding studies comparing growth of both oysters and bay scallops, *Argopecten irradians*, on the high-lipid *Tetraselmis* strains and other algal strains used widely in molluscan hatcheries. Five *Tetraselmis* strains were found to support oyster growth that was significantly and appreciably more rapid than other algal diets when fed at an equivalent ration. The three of these strains that we tested with post-set scallops were superior diets for this mollusk as well. Sizes of the high-lipid *Tetraselmis* cells range from 9–15 mm; therefore they are not suitable for first-feeding larvae, but some may be useful as diets for older larvae. Algal division rates, temperature tolerance, and effects of growth conditions on nutritional value remain to be investigated. Nevertheless, we believe that these strains offer great potential as diets for hatchery and nursery culture of bivalve mollusks.

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# BIVALVES THAT "FEED" OUT OF WATER: PHOTOTROPHIC NUTRITION DURING EMERSION IN THE GIANT CLAM, *TRIDACNA GIGAS* LINNÉ

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**ABSTRACT** Growth rates of giant clams, *Tridacna gigas* (L.), in intertidal culture suggest that they may be obtaining nutrition from their symbiotic algae despite periods of emersion. This was investigated by measuring rates of photosynthesis and respiration of clams in water and air by respirometry. It was found that the clams can maintain substantial phototrophy in air. However, aerial photosynthesis-irradiance (P-I) curves had lower initial slopes ( $\alpha$ ) and reduced maximum photosynthesis rates ( $P_m$ ) compared to aquatic P-I curves. This was presumably due to the clam's mantle tissue collapsing within the valves without support, thus reducing the mantle area and number of zooxanthellae exposed to light. Even so, the contribution of zooxanthellae to total clam respiration (CZAR) was estimated to be about 119% during aerial photosynthesis. Thus, apart from other unique features, *T. gigas* differs from other filter-feeding bivalves in not apparently having to rely on energy reserves during periods of emersion.

**KEY WORDS:** *Tridacna gigas*, phototrophy, respiration, zooxanthellae, CZAR, cultivation, emersion

## INTRODUCTION

Giant clams (Family Tridacnidae) have recently been studied for their mariculture potential. They are one of 2 groups of bivalves harboring the endosymbiotic dinoflagellates, *Symbiodinium* spp., commonly called zooxanthellae (Kawaguti 1983). Thus, giant clams obtain nourishment in several ways: 1) phototrophy (by the transfer of photosynthate from zooxanthellae housed extracellularly within the mantle tissues in a unique diverticulum of the digestive tract); 2) possible digestion of some of the zooxanthellae that pass into their digestive tract; 3) filter-feeding; and 4) uptake of dissolved inorganic and organic molecules (Fitt 1993, Lucas 1994).

In phototrophic nutrition, zooxanthellae translocate to the host up to 95% of the carbon fixed daily (Fitt 1993). This distinguishes tridacnids from other filter-feeding bivalves because, by virtue of their symbiosis, tridacnids require light for survival and growth. In *Tridacna gigas* (L.), the relative dependence on light compared to filter feeding increases with size (Klumpp et al. 1992); while filter feeding in *T. tevoroa* (Lucas et al. 1989) and *T. derasa* (Röding) was found to be relatively unimportant compared to phototrophic nutrition (Klumpp and Lucas 1994). Hence, tridacnid cultivation methods employ single-layered culture systems, unlike the multi-layered cultivation methods frequently used with bivalves.

*T. gigas* may inhabit very shallow to subtidal sunlit waters. In some reefs, this species has been found even in areas exposed to low water (Hester and Jones 1974). By far the largest species of giant clam, intertidal culture has been used successfully for *T. gigas* in the nursery and grow-out phases of tridacnid culture. Furthermore, Lucas et al. (1989) reported a positive correlation between growth of *T. gigas* (based on shell increment) and daytime emersion periods of up to 3 hr; although there was a negative correlation between growth and night emersion period. They hypothesized that "some photosynthesis continues in air due to very high incident light levels on the withdrawn mantle tissue. . . . [so] . . . that giant clams continue to gain energy and nutrients during

emersion." The phenomenon of aerial photosynthesis in aquatic symbiotic organisms is not without precedence. For example, it has been described in the sea anemone *Anthopleura elegantissima* with zooxanthellae still photosynthesizing during emersion (Shick and Dykens 1984).

The aims of this study were to determine if aerial photosynthesis occurs in *T. gigas*, and, if it does, to determine its importance in clam nutrition by estimating zooxanthellar contribution to total clam respiration (CZAR) during emersion. In other words, is phototrophy during emersion a significant component of the energy budget of *T. gigas*?

## MATERIALS AND METHODS

Aquatic and aerial rates of photosynthesis (P, oxygen production in the light) and respiration (R, oxygen consumption in the dark) were measured with 5 juvenile *T. gigas* about 110-115 mm shell length using standard respirometry techniques (Forstner 1983, Mingo-Licuanan 1993), as described below.

P and R were alternately measured with a PHM72 Mk2 Acid-Base Analyzer equipped with a glass oxygen electrode (Radiometer E5046-0), and continuously plotted on a chart recorder. Aquatic P and R were determined with the clam immersed in 0.45  $\mu$ m filtered seawater (33 ppt Sal.) at 27°C  $\pm$  1°C in a hermetically sealed chamber made of ordinary Plexiglass, either 322 or 737 mL volume depending on the size of the clam. A stirrer bar maintained constant water circulation around the oxygen electrode. Oxygen levels of the filtered seawater medium were maintained above 75% saturation level. Intense illumination of the clam mantle tissues for P measurements was provided from a slide projector (Prado 250/500, Leitz Wetzlar). The relationship between P and light intensity (I) was determined by using screens to achieve various irradiances ranging from 40 to 1,670  $\mu$ E  $\cdot$  m<sup>-2</sup>  $\cdot$  s<sup>-1</sup>. Light intensity or irradiance was measured at the chamber surface with a Li-Cor light meter and an underwater quantum sensor (with cosine correction optimized for both underwater or atmospheric use). Irradiance was altered at random to avoid any conditioned response. The chambers were in total darkness to measure R.

Aerial P and R for the same clams were later determined in the same chambers using the same system of illumination. During these measurements, a solution of diethanolamine contained in a

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vial served as a reversible CO<sub>2</sub> buffer, capable of 95% absorption and maintaining an atmospheric CO<sub>2</sub> level of 3%. This buffer ensures that atmospheric CO<sub>2</sub> is not depleted with continued photosynthesis (Pardee 1949, Krebs 1951, Umbreit 1964). For aerial P, irradiances used ranged from 60 to 1,000  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . The reason for not measuring aerial P beyond 1,000  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  was to lessen any clam distress since preparing the clams for emersion measurements took longer than immersion measurements. Emersion preparations included positioning the clam in a standard manner to expose its mantle to light, ensuring that the clam expelled its remaining mantle cavity water, etc.

CZAR was estimated by the equation (Muscatine et al. 1981):

$$\text{CZAR} = \text{P/R} \times \text{T}\%$$

where P/R is the ratio of net photosynthesis to respiration, calculated from the equation (Muscatine et al. 1981):

$$\text{P/R} = \frac{(\text{P}^\circ \times \text{tday})(0.375\text{PQ}^{-1}) - (1 - \beta)(\text{R}^\circ \times 24)(0.375\text{RQ})}{(\beta)(\text{R}^\circ \times 24)(0.375\text{RQ})}$$

where P<sup>°</sup> is gross oxygen production at saturating light intensity; tday is the period during which the clam was under saturating light intensity; PQ is the photosynthetic quotient; R<sup>°</sup> is the average R of the intact clam;  $\beta$  is the host contribution to total respiration; (1- $\beta$ ) is the algal contribution to total respiration; and RQ is the respiratory quotient. T% is the translocation efficiency, i.e., the percentage of photosynthetically fixed carbon released by zooxanthellae to the host. Respiration data for the 5 clams used in this study were included in the data set of Mingo-Licuanan (1993).

CZAR was calculated with the following assumptions: 1) rates of day and night respiration are equal; 2) respiration continues throughout each 24-hour period; 3) PQ is equal to 1.1; 4) RQ is equal to 0.8 (the logic of these first assumptions are explained by Muscatine et al. 1981); 5) total clam respiration is composed of 5% algal and 95% host respiration (Trench et al. 1981); 6) T% is 95% (Fitt 1993, Klumpp and Lucas 1994). P-I curves were estimated by non-linear regression (BMDP3R Statistical Program, Health Sciences Computing Facility, University of California, Los Angeles, CA) based on the hyperbolic tangent function (Jassby and Platt 1976, Chalker 1980).

All measurements were conducted at approximately 30 minute intervals, between 7 a.m. and 7 p.m. Each clam was dissected after each complete set of aquatic and aerial measurements to obtain clam wet tissue weight (WTW). The clam mantle was excised and homogenized to extract the zooxanthellae, and hence the number of zooxanthellae  $\cdot \text{clam}^{-1}$  was determined (Mingo 1988).

## RESULTS AND DISCUSSION

Figure 1A and B show gross photosynthetic rates based on clam WTW and on number of zooxanthellae per clam (expressed as per alga) versus irradiance (1). Table 1A and B show aquatic and aerial photosynthetic parameters, P<sub>m</sub>, I<sub>k</sub> and  $\alpha$ , derived from these sets of P-I curves. Such that:

$$\alpha = \frac{\text{P}_m}{\text{I}_k}$$

where  $\alpha$  is the initial slope of the P-I curve and I<sub>k</sub> is where  $\alpha$  intersects with P<sub>m</sub>.

The clams showed significant photosynthesis during emersion. However, on a WTW basis, immersed clams had much higher

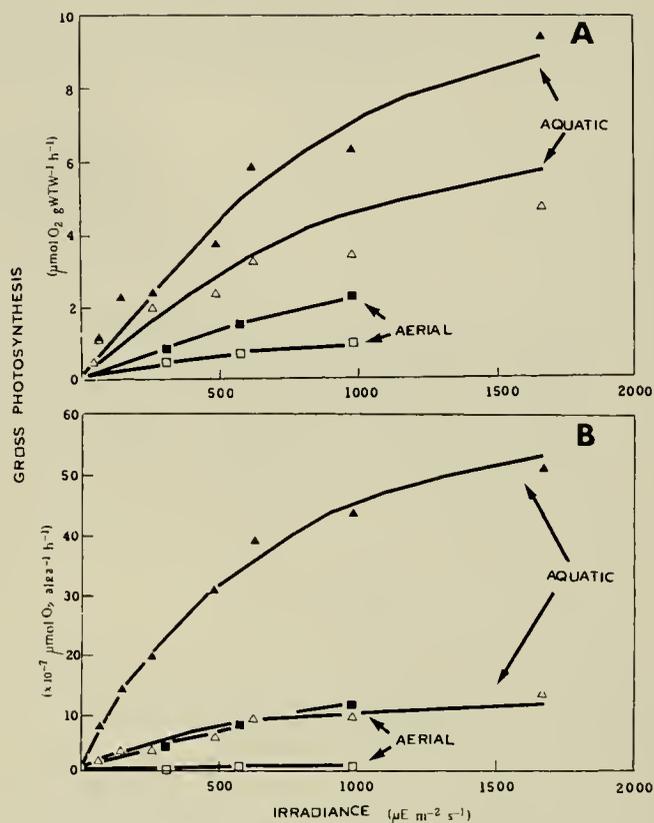


Figure 1. Aquatic and aerial photosynthesis rates of intact *Tridacna gigas*, based on (A) clam wet tissue weight (per WTW) and (B) number of zooxanthellae (per alga). Minimum and maximum P-I curves are shown.

values for  $\alpha$  (Paired-sample t test,  $t = 11.087$ ,  $v = 4$ ,  $P < 0.001$ ) and P<sub>m</sub> (Paired-sample t test,  $t = 5.767$ ,  $v = 4$ ,  $P < 0.005$ ). These mean that both the initial slope of the P-I curve with increasing light intensity and the maximum rate of photosynthesis at saturating irradiance were much lower during emersion. Aerial values for  $\alpha$  and P<sub>m</sub> and based on WTW were 8–25% and 9–16%, respectively, of the aquatic values. On an algal basis, P<sub>m</sub> was also higher (Paired-sample t test,  $t = 3.803$ ,  $v = 4$ ,  $P < 0.02$ ) for clams in water than in air. The parameter  $\alpha$  showed statistically similar values for aquatic and aerial measurements (Paired-sample t test,  $t = 2.335$ ,  $v = 4$ ,  $P > 0.05$ ), although the data show apparently higher values for clams in water than in air. The I<sub>k</sub> values for the aquatic P-I curves per WTW and per alga and for the aerial P-I curves per alga were similar (2-sample t test,  $t = 0.453$ ,  $v = 8$ ,  $P > 0.50$ ), within the variability of the data, the mean values being within  $\pm 1$  S.D. of each other. The I<sub>k</sub> values for aerial P-I curves based on WTW could not be predicted or estimated, for lack of further readings beyond 1,000  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Nonetheless, the P-I curves for aerial measurements which were fitted by eye, show that I<sub>k</sub> must be a value greater than 1,000  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

P/R ratios and CZAR values for immersed and emersed clams are shown in Table 2. Thus, for juvenile *T. gigas* of about 110 mm shell length, mean CZAR during immersion and emersion was about 250 and 120%, respectively. Aerial CZAR values are significantly lower than aquatic values (2-sample t test,  $t = 4.124$ ,  $v = 8$ ,  $P < 0.005$ ).

These results show that aerial photosynthesis occurs in *T. gigas*

TABLE 1.

Aquatic (A) and aerial (B) photosynthetic parameters from P-I curves of *Tridacna gigas*, based on wet tissue weight (= per WTW), and number of zooxanthellae per clam (= per alga).

Clam No.	A. During Immersion					
	Per WTW			Per Alga		
	$P_m^a$	$Ik^b$	$\alpha (\times 10^{-3})$	$P_m^c (\times 10^{-7})$	$Ik^b$	$\alpha (\times 10^{-10})$
1	5.94	755	7.87	17	790	21.52
2	4.37	706	6.19	16	740	21.62
3	6.74	634	10.63	54	520	103.85
4	5.75	643	8.94	35	700	50.0
5	9.62	1,053	9.15	13	850	15.29
Mean	6.64	778	8.54	27	720	42.46
S.D.	1.79	161	1.47	17	125	36.87

Clam No.	B. During Emersion					
	Per WTW			Per Alga		
	$P_m^a$	$Ik^b$	$\alpha (\times 10^{-3})$	$P_m^c (\times 10^{-7})$	$Ik^b$	$\alpha (\times 10^{-10})$
1	1.28	>1,000	1.28	3.40	432	7.87
2	0.85	>1,000	0.85	0.74	840	0.88
3	1.48	>1,000	1.48	11.40	890	12.81
4	1.45	>1,000	1.45	7.90	430	18.37
5	0.8	>1,000	0.80	1.30	735	1.77
Mean	1.17	>1,000	1.17	4.95	665	8.34
S.D.	0.33		0.33	4.58	221	7.41

$\alpha = P_m/Ik$ .

<sup>a</sup> In units of  $\mu\text{mol O}_2 \cdot \text{g WTW}^{-1} \cdot \text{h}^{-1}$ .

<sup>b</sup> In units of  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

<sup>c</sup> In units of  $\mu\text{mol O}_2 \cdot \text{alga}^{-1} \cdot \text{h}^{-1}$ .

during emersion. They do photosynthesize out of water. This must be because emersed clams do not close their valves completely, allowing solar radiation to impinge on exposed mantle tissue (see cover photograph). However, lack of support for the mantle causes its collapse back inside the shell, thus reducing the area of mantle and the number of zooxanthellae receiving light exposure (Mingoa-Licuanan 1993). The reduced area of exposed mantle presumably causes the lower slope of the P-I curve ( $\alpha$ ) and lower maximum photosynthetic rate ( $P_m$ ) in emersed (or air-exposed) clams, compared to immersed clams (Table 1).

Notwithstanding the substantial reduction in aerial photosynthetic rate (Fig. 1), the CZAR estimates indicate that, for a 110 mm juvenile *T. gigas* (about 33 g wet flesh weight), phototrophy at saturation light levels ( $P_m$ ) supplies about 119% of the carbon requirements that could be used for clam metabolism during exposure in air (Table 2). This is because clam respiration is also reduced in air; to about a third of its rate in water (Mingoa-

TABLE 2.

P/R ratios and CZAR values (%) for *Tridacna gigas* in water and in air.

	In Water		In Air	
	P/R	CZAR (T = 95%)	P/R	CZAR (T = 95%)
	1.67	159	1.16	110
	2.18	207	1.10	105
	3.42	325	1.50	143
	2.96	282	1.42	135
	3.41	324	1.06	101
Mean	2.73	259	1.25	119
S.D.	0.78	74	0.20	19

Licuanan 1993). If no anaerobic metabolism is occurring, phototrophy is quite sufficient to support the clam's metabolism during periods of emersion.

When juveniles of *Tridacna gigas* are emersed and placed in the dark, they develop oxygen debts (Mingoa-Licuanan 1993), as would be expected; there are no equivalent data for clams emersed in light. If subsequent studies show that they do not develop oxygen debts when emersed in  $P_m$  light conditions, this would be good support for the validity of the CZAR value found in this study.

These results support the hypothesis of Lucas et al. (1989) that because moderate periods of emersion during the day (but not night) promote growth, cultured *T. gigas* must be photosynthesizing even when exposed to air. Furthermore, other tridacnid species inhabit the intertidal zone, especially *T. crocea*, *T. maxima* and the 2 *Hippopus* species, and it is likely that they also show degrees of phototrophy during emersion. They all show valve gaping and some extent of mantle exposure within the valves. This further distinguishes tridacnids from other filter-feeding bivalves (Helinga and Fitt 1987). Whereas other bivalves have to draw on energy reserves to meet the metabolic cost of maintenance during emersion, intertidal tridacnids are assured of at least some energy input through zooxanthellar photosynthesis.

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## THE REPRODUCTIVE CYCLE OF THE TUATUA-PAPHIES SUBTRIANGULATA (WOOD, 1828), IN NEW ZEALAND

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**ABSTRACT** The reproductive cycle of the tuatua, *Paphies subtriangulata* (Wood 1828), a shallow-water surf clam, was studied from January to November 1993 in Little Omaha Bay, north-eastern New Zealand. Monthly samples were obtained from a shallow sub-tidal population, and analysed by microscopic examination of histological sections of gonad. Four gametogenic stages best described the annual reproductive cycle. Three distinct phases of the reproductive cycle were identified: spawning from February to April, regeneration of gonad from May to late August and resumption of spawning activity from September to November. Gonads routinely exhibited several stages simultaneously and showed a slow progression in the dominance of these stages. Observations of natural spawning events in the field were made on eight occasions and verified the spawning times deduced from histological sections. These observations indicated that only a small proportion of the population spawned at one time, that there were large variations in spawning activity on a daily basis, and that the co-occurring related species *Paphies australis* spawned synchronously with *Paphies subtriangulata*. A 1:1 sex ratio was determined for adult tuatua and no hermaphrodites were encountered.

**KEY WORDS:** Bivalve, New Zealand, *Paphies subtriangulata*, surf clam, reproductive cycle, histology, spawning

### INTRODUCTION

The tuatua is the most inshore of New Zealand's surf clam species, occurring in depths of up to 4 metres (Cranfield et al. 1994). The colloquial term "tuatua" is applied to 2 separate species as defined by Richardson et al. 1982, which co-occur on the same beaches in central New Zealand. Beu and DeRooij-Schuling (1982) classified the 2 species as *Paphies subtriangulata* (Wood 1828) and *Paphies donacina* (Spengler 1793) and summarised their distribution patterns. *P. subtriangulata* is found mainly around the North Island and along the north coast of the South Island, while *P. donacina* occurs around the South Island, the north coast of Stewart Island, and around the lower part of the North Island. The 4 New Zealand *Paphies* species: the pipi, *P. australis*, the toheroa, *P. ventricosa* and the tuatua *P. subtriangulata* and *P. donacina* are highly prized as a recreational resource (Redfearn 1987). The tuatua's extensive distribution around New Zealand combined with large localised populations provides a moderate economic resource. Reproductive cycles of New Zealand surf clam species have received minimal examination to date. Recent interest in harvesting New Zealand surf clams (Smith et al. 1989, Cranfield and Michael 1992) has accelerated the need for information on their reproductive biology (i.e., the recruitment potential of stocks).

Condition indices are often used for commercial bivalve species such as oysters (Muniz et al. 1986) and mussels (Hickman and Illingworth 1980, Emmett et al. 1987), as they provide a useful means of assessing the overall health of a population and hence its suitability for harvest (Crosby and Gale 1990). These indices rely on changes in total body size or weight (Dupaul et al. 1989) and are not direct measures of changes occurring within the gonad (i.e., they may indicate a change in the utilisation of a food source). Thus, although they may provide an indication of when spawning has occurred (Giese 1959) (i.e., a drop in the condition index due to decreased gonad size/weight), they are insufficient descriptors of the reproductive cycle. Dickie (1986) and Hooker and Creese (1995) found that integration of gonad and somatic tissue prevented the use of gonad indices when studying the re-

productive cycle of the pipi. This amalgam of gonad and viscera is also found in *P. subtriangulata* and precluded the use of gonad indices. Consequently, histological sections were utilised to describe gonad development.

Histological techniques can be subdivided into 2 categories: classification or quantitative staging methods. The former is based on visual assessment of gonad tissue with subsequent classification into particular developmental stages (Shaw 1964, Ropes 1968, Mann 1982, Manzi et al. 1985, Baron 1992). The latter involves enumeration of gametogenic products (i.e., oocytes or ova) either by staging a defined number of oocytes (Robinson and Breese 1982, Hadfield and Anderson 1988), by estimating the total number of oocytes per individual (Brousseau 1978), by the percentage of the lumen filled with oocytes (planimetry, Eversole et al. 1980), or by oocyte size/frequency (Keck et al. 1975, Grant and Tyler 1983, Heffernan et al. 1989, Kanti et al. 1993).

Early studies of reproduction in the genus *Paphies* in New Zealand utilised condition indices and only recently have detailed, histological techniques been used for *P. australis*; no such investigations have been carried out for *P. subtriangulata* (Table 1). A single study of tuatua reproduction has been conducted on the west coast of the North Island (Greenway 1981), but ours is the first description from the warmer waters of New Zealand's north-eastern coast (Figure 1).

This paper examines the reproductive cycle of the tuatua (*P. subtriangulata*) by histological classification of gonad sections. An additional objective was to substantiate any suspected spawning peaks with actual observations of spawning in the field, something that has not previously been done for surf clams.

### MATERIALS AND METHODS

#### Collection Procedure

The reproductive cycle of *P. subtriangulata* was studied by sub-tidal sampling. Specimens were collected by SCUBA in a water depth of 1–3 metres, from the southern site in Omaha Bay (Fig. 2), from January to November 1993. Dates of collection

TABLE 1.  
Previous studies of reproductive cycles of the genus *Paphies* in New Zealand.

Species	Author (Site)	Methodology
<i>P. ventricosa</i>	Rapson 1952 (Dargaville)	● Condition Index (wet weight of edible meat/whole animal)
	Redfearn 1974 (Dargaville)	● Condition Index (% of the volume of the shell cavity occupied by meat)
<i>P. subtriangulata</i>	Greenway 1981 (Dargaville)	● Histology of gonad sections
<i>P. australis</i>	Booth 1983 (Bay of Islands, Raumati Beach, Wellington Harbour)	● Condition Index (% of the volume of the shell cavity occupied by meat)
	Dickie 1986 (Whangarei Harbour)	● Condition Index (weight of flesh (g)/weight of flesh + shell (g) × 100)
	Hooker and Creese 1995 (Whangateau Harbour)	Larval occurrence ● Histology of gonad sections ● Histology of gonad sections

The location of these sites is shown in Figure 1.

were largely determined by high tide times and calm weather conditions, but were as close as practical to monthly intervals. Specimens were measured for shell length and the first 30 individuals with a shell length greater than 50 mm were selected. Individuals of this size consistently contained gametogenic material (Grant and Creese unpubl. data). Over the 11-month period of field sampling, a total of 330 tuatua, with shell lengths ranging from 50–70 mm were collected and examined.



Figure 1. Map showing the locations of previous reproductive studies of New Zealand *Paphies* species. See Table 1 for further details.

#### Sex Ratio

Male and female tuatua are externally indistinguishable and can only be reliably sexed by microscopic examination of gonad smears. Sex ratios were obtained in this manner for each monthly sample during preparation of samples for histological analysis.

#### Histological Preparation

Specimens were processed by removing the viscera from the siphons, gills and mantle, and were subsequently fixed in Bouins solution for 48–72 hours, before storage in 70% ethanol to await

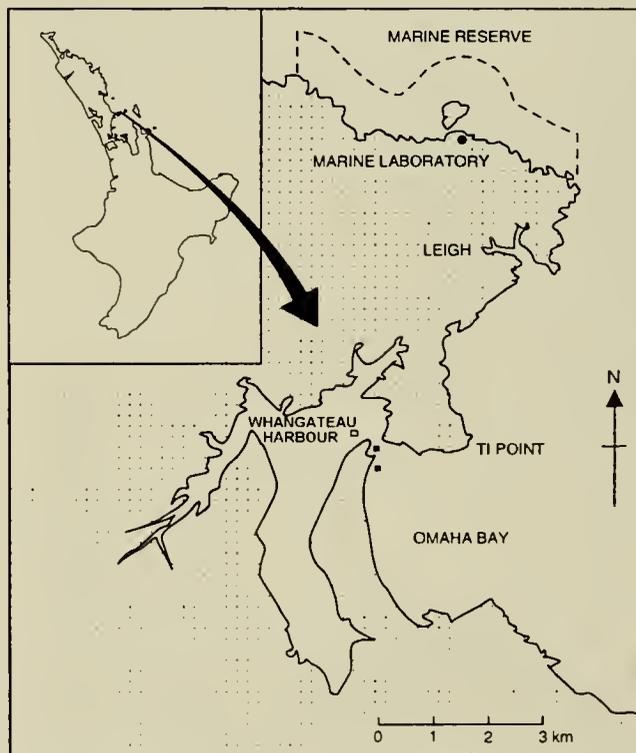


Figure 2. The two sampling sites for *P. subtriangulata* (black boxes), north-eastern New Zealand. The open box indicates the site of the submersible data logger in the adjacent estuary.

sectioning. Examination of gonad variability in *P. australis*, showed no significant difference in reproductive state, between 8 random sections, taken from different positions in the gonad, for either sex (Dickie 1986). Dickie concluded that variation within a section was greater than variation between sections. Consequently, we restricted our sampling to 2 standard mid-lateral gonadal tissue samples from the visceral mass (Hooker and Creese 1995). Tissue samples were then dehydrated in an ethanol series, blocked in paraffin wax, sectioned at 7–10  $\mu\text{m}$ , stained using Ehrlich's Hematoxylin, counterstained with Eosin, and mounted on microscope slides.

#### Histological Staging Criteria

Slides were initially examined under low power (40 $\times$ ) to scan the entire gonadal area, and then again under high power (200 $\times$ ) to assess random follicles. Sections were assigned to one of 4 stages: 1) early active, 2) late active, 3) ripe and 4) partially spawned to spent stage, based on the staging criteria used by Redfean (1974). These categories are only approximations of gonadal development, as distinct stages were not always clear (Ropes 1968). Repeated viewing of the total sample (330 slides) allowed staging criteria to be checked and refined. Two or more stages often occurred simultaneously within each section, therefore staging criteria decisions were based upon the condition of the majority of the section.

#### Temperature

Surface seawater temperature (SST) was measured daily at the Leigh Marine Laboratory (8.5 km from the study site). A submersible data logger (SDL, model 606) was also used on one occasion to obtain *in situ* measurements of seawater temperature, in the nearby Whangateau Harbour (Fig. 2). The SDL recorded the temperature every 30 minutes for a 50-hour period.

#### Natural Spawning Observations

On each monthly sampling occasion of tuatua at the southern site in Omaha Bay, a visual search was made for any spawning activity. Additional observations were also made at a second site 400 m to the north of the main population (Fig. 2). When spawning activity was detected the following observations were made: state of the tide, duration of the observation, approximate number of individuals observed spawning, water temperature and any additional information (e.g., behavioural changes, other species spawning).

## RESULTS

#### Sex Ratio

*P. subtriangulata* are gonochoristic with no evidence of hermaphroditism found. One case of parasitism by trematodes was found in the August sample, causing inability to stage that particular gonad, but the individual could still be reliably sexed as a male. A month-by-month analysis (Table 2) indicated that the sex ratio was not significantly different from 1:1 ( $\chi^2$  test,  $P > 0.05$ ) for any of the 11 samples. The overall sex ratio for *P. subtriangulata*, determined from the total number of individuals sampled, was not significantly divergent from 1:1 (Table 2).

#### Gametogenic Cycle

The gametogenic cycle of *P. subtriangulata* can be described by 3 main phases: 1) an initial spawning period from February to

TABLE 2.

Sex ratios from monthly gonad samples of *Paphies subtriangulata* (N = 30) collected from Omaha Bay, north-eastern New Zealand, January to November 1993.

Months	Females	Males	F:M Ratio	$\chi^2$
Jan	16	14	1.1:1	0.13
Feb	14	16	0.88:1	0.13
Mar	14	16	0.88:1	0.13
Apr	17	13	1.3:1	0.53
May	17	13	1.3:1	0.53
Jun	19	11	1.7:1	2.13
Jul	13	17	0.76:1	0.53
Aug	14	16	0.88:1	0.13
Sep	14	16	0.88:1	0.13
Oct	16	14	1.1:1	0.13
Nov	13	17	0.76:1	0.53
Total:	167	163	1.01:1	0.048

April, 2) regeneration of gametes from May to late August, and 3) a second spawning period from September to November (Fig. 3). This pattern suggests a reproductive cycle commencing in the austral spring (September) and continuing through to the late summer (April). Spawning was comparatively synchronous with respect to sex, but males (Fig. 3A) were slightly more advanced than females (Fig. 3B). No undifferentiated individuals were encountered during the course of this study.

In January both sexes were in either the late active or ripe stage. By February, 50% of the males and approximately 30% of the females were in the partially spawned stage. The presence of some early active stage females indicated regeneration of gonadal tissue post spawning, and correlated well with an observed spawning event on 4 February 1993, the gonad sample being collected 3 weeks post spawning on 28 February 1993. By mid March, 93% of the males and 75% of the females had partially spawned, with the remainder of the population in the late active or ripe stage. The April sample again showed that a large proportion of the popula-

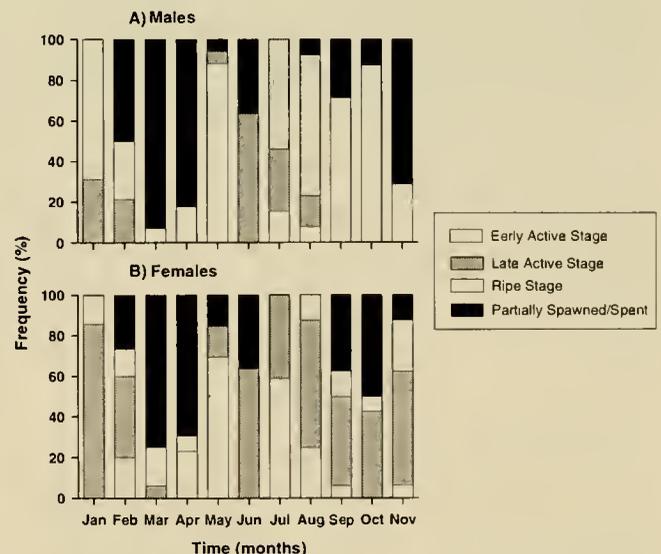


Figure 3. Gonad developmental stages (% of specimens) of *Paphies subtriangulata*, in Omaha Bay (January to November 1993) based on four gonadal stages for (A) males and (B) females.

tion had partially spawned, while some of the females had begun to regenerate. Over this initial spawning period the majority of individuals were considered to have partially spawned as large numbers of mature gametes remained in the gonad.

There was a strong tendency towards regeneration of gonadal material in May, with approximately 78% of the total population in the early active stage. However, a few partially spawned individuals were encountered. June showed a progression to late active stage individuals in both males and females; males were again slightly more advanced with some ripe (stage 3) and partially spawned (stage 4) individuals. The July sample was similar to June but did not contain any spawned individuals. August showed a progression to the ripe stage, although the females lagged behind the males (with more in the late active stage).

Spawning began again in September with approximately 35% of the population in the partially spawned phase. The October sample revealed that 50% of the females had partially spawned, but only approximately 12% of the males. The remainder of the males were still in the ripe stage. November showed the reverse of this with more males than females in the partially spawned to spent stage. A third of the population were still in the ripe phase, mature and ready to spawn. Field observations of spawning events in October and November again confirmed histological staging results. This spring spawning period produced several totally spawned individuals. However, in comparison to the late summer spawning, a smaller percentage of the total population appeared to spawn.

#### Temperature

The mean seawater temperature profile (Fig. 4) exhibits a range in temperature from 13.3°C (August) to 19.7°C (January) over the study period. Spawning was apparently absent over the coldest winter months.

A single 50-hour cycle was obtained using the SDL (Fig. 5), and gives an indication of the daily variation in water temperature at the mouth of the adjacent Whangateau Harbour (Fig. 2). These temperatures can fluctuate by approximately 3.5°C (i.e., half the annual range recorded at the Leigh Marine Laboratory). The SDL temperature profile also demonstrates the effect the combination of time of the day and tide state (high or low) can have on seawater temperature over a particular site. For example, a high tide which occurred during the day has the ability to increase the following

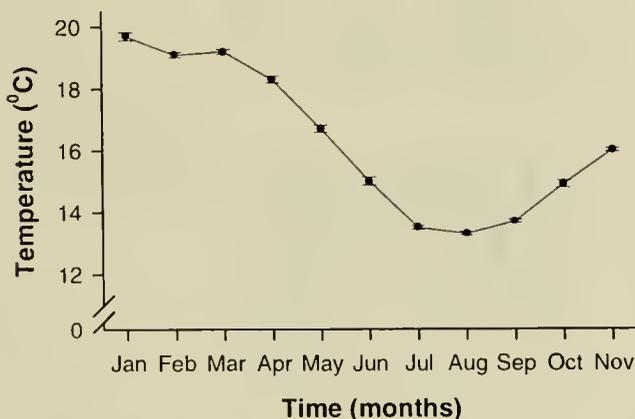


Figure 4. Change in mean surface seawater temperature ( $\pm$ s.e.), measured *ex situ* at the Leigh Marine Laboratory, north-eastern New Zealand (January to November 1993).

low tide seawater temperature, as water which has heated up in the shallower upper harbour rushes out over this site at the harbour entrance (Fig. 2). However, after a high tide which occurred during the late evening or early hours of the morning, the following low tide seawater temperature does not markedly increase, as there is no temperature effect from upper harbour water.

#### Spawning Observations

Spawning was observed on only 8 out of a total of approximately 150 underwater observations (Table 3). Most of the spawning events occurred about 1 hour after an afternoon high tide. An initial spawning observation on 4 February 1993 was detected due to a sudden loss of visibility in the water column above the adult bed. Males customarily began to spawn before females, gametes of both sexes being shed intermittently in thin jet streams from the exhalant siphons. Spawning was not observed again until 21 October 1993, after which an intensive period of spawning continued until the end of observation in November 1993.

On 3 occasions in October, pipi and tuatua were observed to spawn synchronously (Table 3). Initial spawning events were noticed because pipi were uncharacteristically sitting up out of the sand by about 4 centimetres (this was later noted as a common pre-spawning activity in pipi, but was not evident in tuatua). On all 3 occasions, initial spawning activity occurred in the shallower water and then steadily progressed to individuals in deeper water. A clear indicator of spawning activity in both pipi and tuatua was the total extension and full expansion of both the inhalant and the exhalant siphons. Individuals of both pipi and tuatua were observed to spawn sporadically within a single spawning event. On only one occasion was spawning witnessed below a seawater temperature of 15°C.

#### DISCUSSION

Gametogenesis in tuatua appeared to be continuous as no evidence of an inactive or resting stage was observed. Follicular cells were present in several early active stage individuals. These cells are thought to serve functions in nutrition or phagocytosis (Ansell 1961, Porter 1964, Ropes and Stickney 1965), or in expansion of developing follicles (Keck et al. 1975). The latter explanation seems probable for *P. subtriangulata*, as these cells were only apparent in obvious early active stage individuals.

Qualitative staging permitted identification of major changes in the reproductive cycle, but minor fluctuations within each stage could not be distinguished as gametogenesis occurred along a continuum from spawning to regeneration of gonads (i.e., more than one stage was present in each gonad section). The presence of

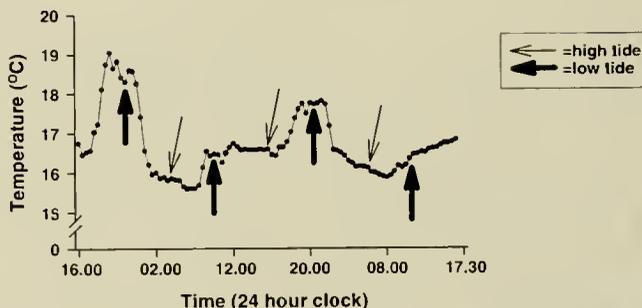


Figure 5. Temperature profile obtained from the Submersible Data Logger from the Whangateau Harbour for a 50-hour period, beginning 23 November 1993 and concluding on 25 November 1993.

TABLE 3.

Spawning observations from Omaha Bay, north-eastern New Zealand. Seawater temperature was recorded *in situ* at the time of each spawning observation.

Date:	Moon:	Tide Phase:	Time:	Tide State:	Number Spawning:	Temperature °C:	Species:
4/2/93	1st quarter	Spring - 3	17:53	High	5-10	18°C	tuatua
21/10/93	1st quarter	Neap - 1	12:46	High	60-80	15°C	tuatua and pipi
22/10/93	1st quarter	Neap	13:39	High	30-40	15°C	tuatua and pipi
26/10/93	1st quarter	Neap + 4	17:23	High	10-15	15°C	tuatua and pipi
2/11/93	full moon	Spring + 2	10:00	High	20	15°C	tuatua
3/11/93	3rd quarter	Spring + 3	16:39	Low	3	14°C	tuatua
9/11/93	3rd quarter	Neap + 2	15:30	High	50+	16°C	tuatua
23/11/93	1st quarter	Neap + 2	15:46	High	25	16°C	tuatua

gametes at all stages of maturity has been linked by previous authors (Shafee and Lucas 1980, Baron 1992) to the occurrence of successive individual spawnings. Overlapping generations of gametes found in this study (i.e., residual ripe oocytes after spawning) and field observations of individuals spawning intermittently during a spawning event corroborate this hypothesis.

Spawning was apparent over most of the study period, as spawned individuals were found in 9/11 months for males and 8/11 months for females. Spawning was frequently incomplete while the 2 most intensive spawning intervals occurred over narrow periods of approximately 3 months (i.e., February to April and September to November). It is likely that the actual annual pattern involves a longer, continuous period stretching from September in 1 year to April in the following year. *P. australis* also spawns over an extended period during this time of year (Dickie 1986, Hooker and Creese 1995). The spring period (September to November) found in our study corresponds to the much more contracted period suggested by Greenway (1981) for *P. subtriangulata* on the west coast. Greenway's study utilised a condition index which reached low levels in summer months and high levels in spring. When considered along with growth and size frequency data, Greenway (1981) suggested a major spawning phase between October and December.

An equal sex ratio was determined for adult *P. subtriangulata*. Previous studies in the genus *Paphies* have also indicated a 1:1 sex ratio (Dawson 1954, Redfearn 1974, Dickie 1986, Hooker and Creese 1995). Sex ratios within a population may change with respect to age (i.e., protandry). Hooker and Creese (1995) recently showed that juvenile *P. australis* (less than 40 mm in length) have a sex ratio strongly biased towards females. Sex ratios may also vary between different populations of the same species, as Dickie (1986) found for *P. australis*, with individual sites showing up to a 1:3 bias. These findings indicate a need for research between different populations of *P. subtriangulata* before widespread generalisations can be made.

Gamete maturation and spawning in bivalves is mainly controlled by 2 factors; temperature and food supply (Brey and Hain 1992). A relationship between the reproductive cycle and food availability has been demonstrated by several authors (Hadfield and Anderson 1988, Shafee 1989), and is thought to ensure adequate nutrition for developing planktonic larvae. However, temperature is considered the main environmental cue for induction of gametogenesis and spawning in temperate regions (Mann 1979, Hadfield and Anderson 1988, Harvey and Vincent 1989). *P. subtriangulata* stopped spawning over the coldest winter months

(Figs. 3 and 4). In addition, observed spawning events commonly occurred about 1 hour after an afternoon high tide when warmer water was beginning to leave the adjacent estuary, and on only one occasion was spawning observed on a low tide (3 November 1993). A minimum temperature threshold is also suggested by the fact that spawning was largely limited to occasions when water temperature was above 15°C. *In situ* evidence gained from the adjacent mouth of the Whangateau Harbour (Fig. 5) revealed the range in temperature that may occur over a 2-day period. Baron (1992) suggests that intertidal organisms are exposed to much sharper daily temperature fluctuations in summer months, and that these fluctuations of variable magnitude may well trigger spawning in bivalves. A detailed investigation of the role temperature plays as a spawning cue is warranted from the preliminary results gathered to date.

Most events in the gametogenic cycle can only be indirectly inferred from examination of the gametogenic tissue. Spawning, the culmination of the entire gametogenic cycle, however, can be directly observed in the field. In an attempt to more accurately describe the temporal component of the gametogenic cycle, we made extensive *in situ* observations of spawning behaviour or the absence of it.

These observations also allowed assessment of variations in spawning between adjacent populations, between individuals within a population, and between co-occurring tuatua and pipi. To our knowledge this has never been done before for surf clams or other infaunal bivalves. Spawning observations lead to the conclusion that *P. subtriangulata* at the 2 Omaha sites (Fig. 2) spawned in patches. Individuals were observed to spawn more than once within the same spawning event which often lasted for up to 2 hours. We conclude that, for bivalve species such as *P. subtriangulata* which spawn sporadically over an extended breeding season, details of the pattern of spawning and the proximate cues for this, are best examined by *in situ* observation wherever possible.

#### ACKNOWLEDGMENTS

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## REPLENISHMENT OF HARD CLAM STOCKS USING HATCHERY SEED: COMBINED IMPORTANCE OF BOTTOM TYPE, SEED SIZE, PLANTING SEASON, AND DENSITY

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**ABSTRACT** Despite the necessity of intensive management of aquaculture operations to minimize potentially high predation on juvenile hard clams, *Mercenaria mercenaria* (Linnaeus, 1758), aquaculture may provide a feasible means of restocking clam populations on public bottom. We tested the feasibility of restoring clams on public bottom in North Carolina by a combination of use of large (14–22 mm) seed clams, late fall/winter (November to January) planting, sparse planting density (1 m<sup>-2</sup>), and proper bottom habitat (shell hash) as well as selection of traditionally productive sites. Clam survivorship to size of legal harvest was somewhat enhanced by planting seed clams in late fall/winter (November to January) instead of late summer (August to October): a difference of 5.8 vs. 3.6% in sand sediments was significant, whereas a difference of 13.1 vs. 12.2% in seagrass was not. Bottom habitat had a much greater influence, with survivorship of 5% in unvegetated sand vs. 12% in seagrass and 8% in unvegetated mud/sand vs. 27% in oyster-shell hash. By adopting a combination of strategies to minimize predation (use of large seed clams, late fall/winter planting, sparse density, and traditionally productive sites in oyster-shell hash habitat), an average of 35% survivorship was achieved over the 14 months from planting until a time when about 12.5% of the seed had reached marketable size. At a cost for seed of \$0.028 each, a planting cost of \$0.002, a survivorship of 35%, and a dockside price of \$0.12–0.20 per clam, reseeded public bottom in this way could yield an expected \$1.40–2.33 in dockside income per dollar invested. This calculation assumes commercial harvest of all surviving clams without augmentation by an economic multiplier effect, the economic benefits of supporting seed clam hatcheries, the benefits of enhanced spawn release into public waters by the planted clams before harvest, or the direct and indirect economic value of enhanced availability of shellfish resources to tourism, retirement, coastal property valuation, and other economic enterprises.

**KEY WORDS:** bioeconomic feasibility, bottom habitat, *Mercenaria mercenaria*, planting density, planting season, seed size, shellfish stock replenishment

### INTRODUCTION

Although the technology for spawning and raising hard clams, *Mercenaria mercenaria*, in the laboratory to provide low-cost seed for planting and growing out on natural bottom has been known and widely publicized for decades (Loosanoff and Davis 1963, Castagna and Kraeuter 1977, 1981, Castagna 1984), most clam aquaculture attempts using seed clams have failed to turn a profit. Probably the major inhibition to establishment of economically viable culture of hard clams has been successful control of predation losses (e.g., Carriger 1959, Menzel et al. 1976, Whetstone and Eversole 1978, Castagna and Kraeuter 1981), with crabs representing probably the most serious problem predator from Cape Cod to Texas (MacKenzie 1977, Virnstein 1977, Manzi et al. 1980, Hines et al. 1990). Profitable aquaculture of hard clams requires a substantial and informed investment of both labor and materials to limit losses to crabs and other predators. Even after preparing the bottom habitat by application of shell or gravel (Castagna and Kraeuter 1977), using mesh covers that are periodically inspected and cleared of small newly recruited predators (Kraeuter and Castagna 1980, Walker 1984), and employing sand bags as side walls to prevent ingress by burrowing crabs (Castagna and Kraeuter 1981), hard clam aquaculture frequently fails because of uneconomically high losses to predators.

In recognition of the bioeconomic difficulty of raising hard clams in field culture even under intensive management, it may seem foolhardy even to contemplate reseeded natural clam beds on public bottom where no management to control predation

will be feasible. Yet intrinsic differences between culture of clams on private shellfish leases and restoration of clam populations on public bottom may allow viable hard clam reseeded operations to proceed on public bottom. For example, clams are ordinarily packed densely on shellfish leases in aquaculture operations so as to maximize the use of the limited lease area, minimize the costs of protective netting, shell hash, gravel, and sand bags, and minimize labor costs during harvest. Clams planted on public bottom can be widely spaced yet still serve to replenish the stock. Because crab predation on bivalves on natural bottom is typically density-dependent (Boulding and Hay 1984, Eggleston et al. 1992, Summererson et al. 1995), the reduction in density of seed may serve to protect enough of them from predation to render seeding of public bottom economically viable. Furthermore, seeding of public bottom is not constrained to a fixed place but can be done in habitats or specific sites where survivorship is known or expected to be highest. Sites made available for lease by aquaculturists are often politically constrained to the poorest natural shellfish habitats. Combining the planting of seed at low density in habitats and traditional sites where survival is best with use of relatively large seed and a late autumn planting season (Eldridge et al. 1979, Peterson 1990) to further minimize losses to predators may render viable the reseeded clam stocks on public bottom.

Here, we provide results of field trials done throughout the entire coastal region of North Carolina to test whether the combination of (1) low-density planting, (2) choice of bottom type and specific sites known as traditional clamming grounds, (3) use of large seed, and (4) planting in late autumn/winter would permit

bioeconomically justifiable replenishment of hard clams on public bottom. We explicitly varied bottom type (shell bottom vs. seagrass vs. unvegetated sand or sand/mud) and planting season (late summer vs. late fall/winter) as well as evaluating the effectiveness of the combination of low density ( $1 \text{ m}^{-2}$ ), bottom habitat and traditionally productive clamming grounds, large seed size (14–22 mm), and late fall/winter planting. While there is evidence that each of these factors separately affects the survival of seed clams, the novelty of this study lies in the simultaneous manipulation of all of these factors in a fashion intended to maximize survivorship, thereby providing a practical test of bioeconomic feasibility of restocking hard clam populations on publically owned and fished bottom.

#### MATERIALS AND METHODS

To test the effectiveness of seeding public bottom with small hard clams, *M. mercenaria*, we planted seed clams at sites on natural bottom in North Carolina during a period of from late summer 1990 to mid-winter 1990–1991 and then sampled the returns in spring of 1992. To produce the required seed clams, we engaged ARC (Aquaculture Research Corporation) of Dennis, MA to spawn adult clams collected from North Carolina, to grow the spawn up in their hatchery and nursery to a size of 14–22 mm in shell length, and to hold the clams in their nursery in Atlantic, NC for delivery on demand during the period of planting. Seed clam size was not a variable in these trials but rather was held constant at this relatively large size (the large majority of seed clams fell in the 16–22 mm range). Prior to planting in the field, the outer shell surfaces of all clams were permanently stained purple with exposure to alizarin red, a calcium stain (Hidu and Hanks 1968). This not only allowed identification of our test seed but also permitted the shell growth since date of planting to be assessed on survivors by measuring the amount of newly deposited, unstained shell growth. In addition to this staining, a single dot of Mark-Tex Corp. paint was applied to each valve of the seed clams used in a subplot at each site where intensive suction-dredge sampling was planned to evaluate individual survivorship (see below).

To test the importance of planting season on survivorship of seed clams on public bottom, we established an experiment in each of 2 bottom habitat types in Carteret County (Fig. 1). We planted seed clams in 4 seagrass sites and in 6 unvegetated sandy sites:

each of the seagrass sites was paired with a nearby sand site in the design with addition of 2 more sand sites (Table 1). Specific sites were selected after extensive discussions with local shellfishermen and fisheries managers to identify localities that did then or had historically supported substantial natural clam production. Planting density was held constant in this experiment at the low level of  $1 \text{ m}^{-2}$ . At each of these sites, 2 separate plantings took place, one in late summer (from 15 August to 24 October 1990) and another in late fall/winter (from 11 November 1990 to 1 February 1991). In the subplots intended for intensive suction-dredge sampling, these late fall/winter clams were marked with a different color of Mark-Tex Corp. paint to distinguish them from the late summer clams at that same site. All clam planting was conducted in water temperatures exceeding  $11^{\circ}\text{C}$  to avoid deaths from cold shock. Sampling to assess survivorship was conducted from 5 December 1991 to 24 April 1992 (Table 1), at times when on average 12.5% of surviving seed clams at each site had just reached the legally minimum size for harvest (2.5 cm in shell thickness). This timing of final sampling thus assessed survivorship to the size of harvest but allowed only minimal opportunity for legal harvest of the experimental clams to interfere with evaluation of the results.

In addition to the experiment conducted to evaluate the effects of varying planting season, we also established a set of trials to assess the effects of varying bottom habitat. These trials employed the same large seed clams (14–22 mm in length) and low planting density of  $1 \text{ m}^{-2}$  as all the other plantings but used only a single planting season, late fall/winter (12 November 1990 to 6 February 1991) (Table 2). Replicate sites were planted in the northern region of the state (in Dare County near Hatteras Inlet and Hyde County near Ocracoke Inlet) in seagrass beds and in unvegetated sandy bottom (Fig. 1). In the central region in Carteret County, replicate plantings were established in 3 types of bottom habitats in 3 different sites (Fig. 1): seagrass beds, unvegetated sandy bottoms, and oyster-shell hash. In the southern region at Virginia Creek-Stump Sound (Onslow and Pender Counties) and Lockwood's Folly River (Brunswick County), replicate sites were planted in 2 bottom habitat types, unvegetated sand/mud and in oyster-shell hash (Fig. 1). Again, specific sites used for this experiment were selected after extensive discussion with local shellfishermen and fisheries managers to identify locations with a history of high clam production. With the exception of sites in Lock-

TABLE 1.

Locations, habitat types, planting dates and final sampling dates for each of the sites used to assess the effect of planting season on survivorship of seed clams in each of 2 bottom habitat types, seagrass and unvegetated sand.

Site	°N. Lat.	°W. Long.	Planting Date		Final Sampling Date	
			Late Summer	Late Fall/Winter	For Summer Plant	For Fall/Winter Plant
<i>Seagrass habitat</i>						
Banks Bay—1	34°38'11"	76°31'39"	10/16/90	1/14/91	3/3–3/24/92	3/3–3/24/92
Bald Hill Bay—1	34°40'21"	76°34'38"	9/12/90	11/6/90	4/3–4/17/92	4/16–4/28/92
N. Middle Marsh	34°42'26"	76°35'50"	10/18/90	12/18/90	12/10/91	12/9/91
Cedar Point	34°40'02"	77°05'18"	10/3/90	12/1/90	2/12/92	2/13/92–2/24/92
<i>Unvegetated sand habitat</i>						
Horsepen Point	34°43'28"	76°27'13"	10/17/90	1/3–1/4/91	4/1–4/7/92	4/1–4/7/92
Banks Bay—1	34°38'08"	76°31'34"	10/16–10/17/90	1/15/91	3/24/92	2/22–3/3/92
Bald Hill Bay—1	34°39'55"	76°34'45"	9/10/90	11/5/90	12/5/91	12/6/91
N. Middle Marsh	34°42'19"	76°36'12"	10/19/90	12/17/90	2/28/92	2/25/92
S. Middle Marsh	34°41'16"	76°37'07"	9/24–9/25/90	11/20/90	1/21/92	2/28/92
Mid-Bogue Sound—1	34°42'26"	76°56'50"	9/19/90	11/27/90	12/12/91	12/13/91

TABLE 2.

Locations, habitat types, planting date and final sampling date for each of the sites established to augment those listed in Table 1 to provide tests of the effects of bottom habitat type on survivorship of seed clams.

Site	Habitat Type	°N. Lat.	°W. Long.	Planting Date	Final Sampling Date
Northern Region					
Hatteras	Sand	35°12'28"	75°45'55"	11/12/90	4/25/92
Hatteras	Eelgrass	35°12'36"	75°46'10"	12/5/90	4/23/92
Hatteras	Eelgrass	35°12'35"	75°46'07"	12/6/90	4/24/92
Hatteras	Sand	35°12'13"	75°45'38"	12/7/90	5/4/92
Hatteras	Sand	35°12'27"	75°46'16"	12/7/90	5/4/92
Hatteras	Sand	35°12'56"	75°45'47"	12/5/90	6/10/92
Ocracoke Island	Eelgrass	35°07'40"	75°57'32"	11/16/90	6/8/92
Central Region					
Banks Bay—2	Sand	34°38'11"	76°31'29"	1/15/91	2/21/92
Bald Hill Bay—2	Eelgrass	34°40'16"	76°34'42"	1/17/91	3/2–3/31/92
Bald Hill Bay—2	Sand	34°39'53"	76°34'36"	1/16/91	2/20/92
S. Middle Marsh	Oyster shell	34°43'27"	76°37'07"	11/19/90	5/19–6/5/92
Mid-Bogue Sound	Oyster shell	34°42'35"	76°56'48"	12/10/90	6/2/92
Cedar Point	Sand	34°39'53"	77°05'16"	12/1/90	5/12/92
Southern Region					
Stump Sound	Sand/Mud	34°28'32"	77°28'41"	1/19/91	5/15/92
Stump Sound	Sand/Mud	34°27'27"	77°31'06"	1/19/91	5/18/92
Stump Sound	Sand/Mud	34°25'58"	77°32'47"	1/23/91	5/13–5/15/92
Lockwood's Folly R.	Sand/Mud	33°55'35"	78°13'12"	2/2/91	5/29/92
Lockwood's Folly R.	Sand/Mud	33°55'34"	78°12'58"	2/2/91	5/28/92
Lockwood's Folly R.	Sand/Mud	33°56'32"	78°13'05"	2/3/91	5/28/92
Lockwood's Folly R.	Oyster shell	33°55'32"	78°12'57"	2/1/91	6/15–6/16/92
Lockwood's Folly R.	Oyster shell	33°56'33"	78°13'03"	2/3/91	6/17/92

These tests used only sites planted in the late fall/winter season.

wood's Folly River, where water quality violations from *Escherichia coli* contamination had closed the area to shellfishing, the sites used in this study were actively clammed by commercial and recreational clambers during the course of the study. Final sampling of the sites used in these trials was conducted in spring between 4 April and 17 June 1992 (Table 2), after an average of 12.5% of the surviving seed clams had grown to legally harvestable size. In the analyses of the effects of habitat and geographic region, appropriate data sets (those that employed the late fall/winter planting) from the contrast of planting season listed in Table 1 were combined with those identified in Table 2 to enhance replication and use all available information.

For all plantings at all sites in this study, planting methods were identical. Seed clams were planted at the low density of 1 clam  $m^{-2}$  over a plot of 2400  $m^2$ . Plot size was somewhat less in some shell-hash sites because of limited area available for planting in a given site. Elevations of the plots varied among sites only over a narrow range from low intertidal to shallow subtidal (<0.6 m depth at low tide). Each plot was marked at the corners using white PVC shielding a steel rebar core and also with floats attached to cement blocks. Systematic planting of seed clams on 1-m centers was achieved by hand planting at positions identified by a grid of ropes marked at 1-m intervals. In addition, 104 individually marked (with color-coded paint dots) clams were planted in a subplot at each site in even more precisely located positions by using four 25-m long weighted lead-lines, marked at 1-m intervals, to place each clam. The positions of the ends of each lead-line were marked with buried steel bars in inverted U-shapes to permit precise relocation for resampling. Information on clam survivorship was derived from resampling the subplot at each site.

Despite the care in identifying the planting locations so pre-

cisely, the natural mobility of the clams and the disturbance activities of clam harvesters during the period of the experiment typically prevent accurate estimation of survivorship in some plantings. We developed a method to overcome this serious sampling limitation. In addition to planting a seed clam at each meter mark along the lead-lines, we also added a single stone of a type alien to this environment and therefore immediately recognizable. The stones were of a size that was just large enough to be retained by a clam rake with teeth at 2-cm spacing. To resample the subplots, we employed a suction dredge with 3-mm mesh sampling bag to sample circular samples of radius 25 cm at each of the 104 positions where seed clams had been planted. The specially painted seed clams and stones were each collected and counted. The recovery of stones was used to control for sampling error and for the intervention of human disturbance and thereby provide an expected number of clams that should have been recovered at each plot if all planted clams had survived and not moved or been moved further than 25 cm. Seed clam survivorship was then calculated for each plot by comparing the numbers of live painted clams recovered to the numbers of stones recovered. This procedure assumes that stones were displaced from inside the 25-cm radius during any clam raking and harvest activities, which seems reasonable. It also assumes that clams of these sizes do not move more than 25 cm, which is not a reasonable assumption but which biases our survivorship estimates downwards (in a conservative direction). In the larger 2400- $m^2$  matrix, stones were added for every second seed clam. Consequently, our estimate of seed clam survivorship may also be biased downwards somewhat if any stone from the larger matrix became displaced during fishing activity and was recovered inside the subplot. Clams originating from outside the subplots could be distinguished by their lack of Mark-

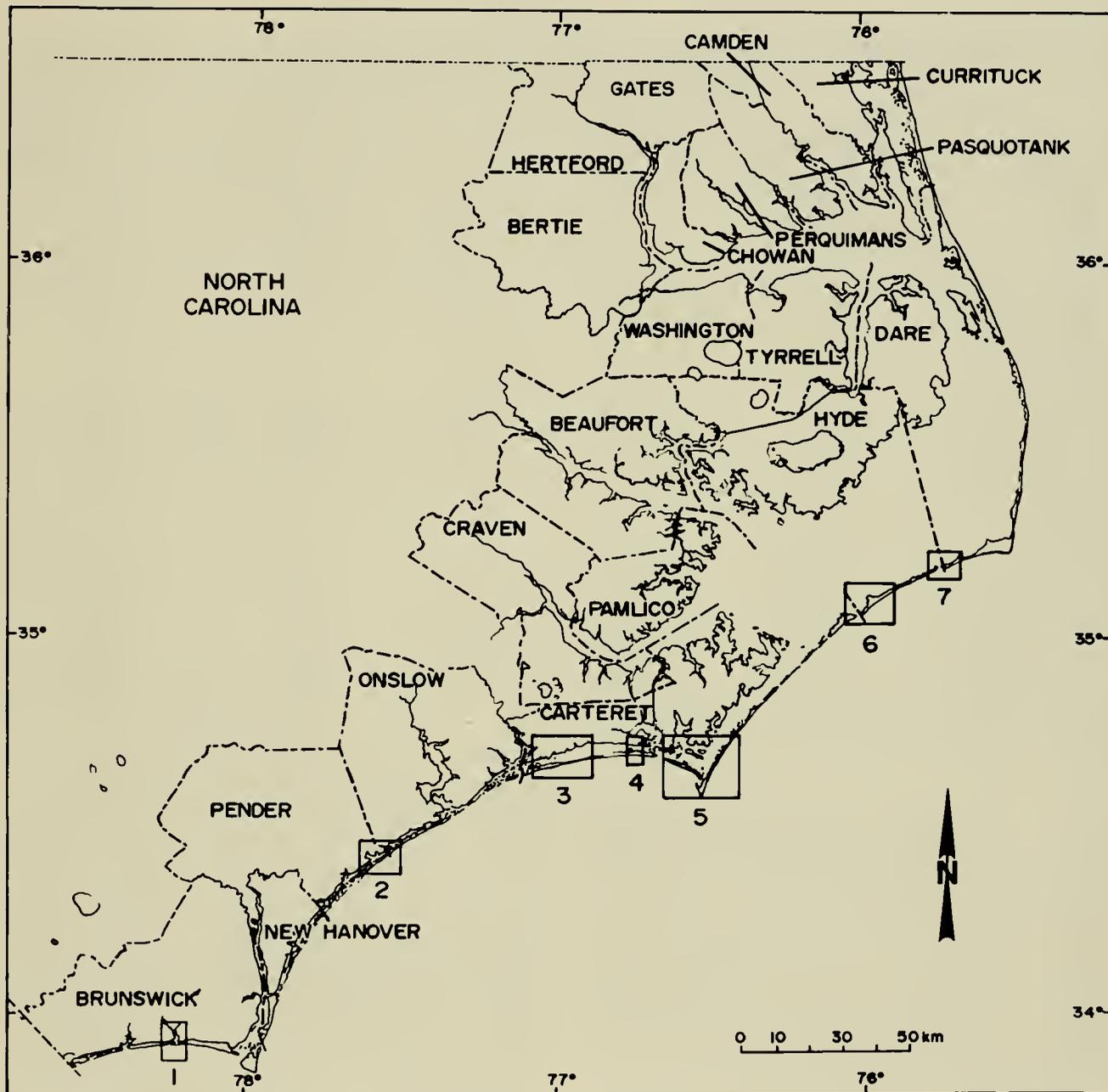


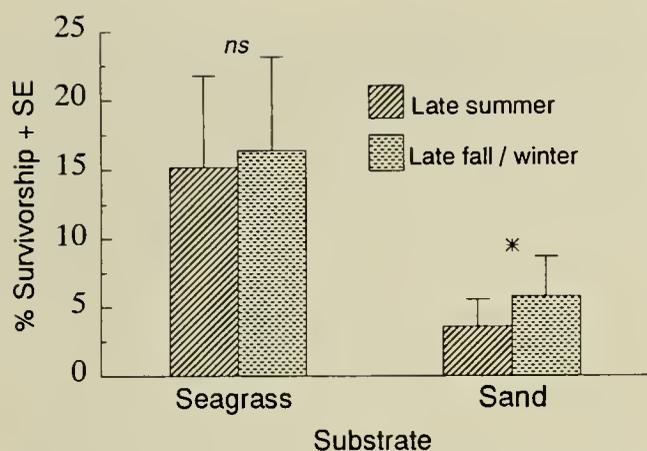
Figure 1. Study sites along the coast of North Carolina used for experimental tests of success of restoring hard clam (*Mercenaria mercenaria*) stocks on public bottom by planting seed clams. Sites from south to north are located in 3 geographic regions: the southern (1, Lockwood's Folly River; 2, Virginia Creek-Stump Sound); central (3, western Bogue Sound; 4, central Bogue Sound; 5, Back and Core Sounds); and northern (6, Ocracoke Inlet; 7, Hatteras Inlet).

Tex Corp. paints but not stones. Care was taken to insert each clam and rock into the sediments during planting so as to minimize the possibility of immediate transport away from the intended planting locus.

## RESULTS

Sampling in winter 1991–1992 for clams planted in either late summer 1990 or late fall/winter 1990–1991 revealed that survivorship was somewhat greater for those clams planted in late fall/winter (Fig. 2). The Wilcoxon signed-ranks test (a test that does not assume normality of distributions, chosen because no trans-

formation adequately homogenized variances) (Sokal and Rohlf 1969) demonstrated that estimated survivorship in the sand habitat was significantly ( $p < 0.05$ ) greater for fall/winter plantings than for late summer plantings by a factor of about 60%, whereas the factor of 10% increase in survivorship from late summer to late fall/winter plantings in the seagrass habitat was not statistically significant ( $p = 0.91$ ). Those clams planted at the late summer planting had been exposed in the field plots for an average of 2.5 months longer (16.5 vs. 14 months) before final sampling than the clams planted at the late fall/winter season (Table 1). About 12.5% of seed clams recovered had reached the legal size for harvest by the time of this final sampling for both late summer and late

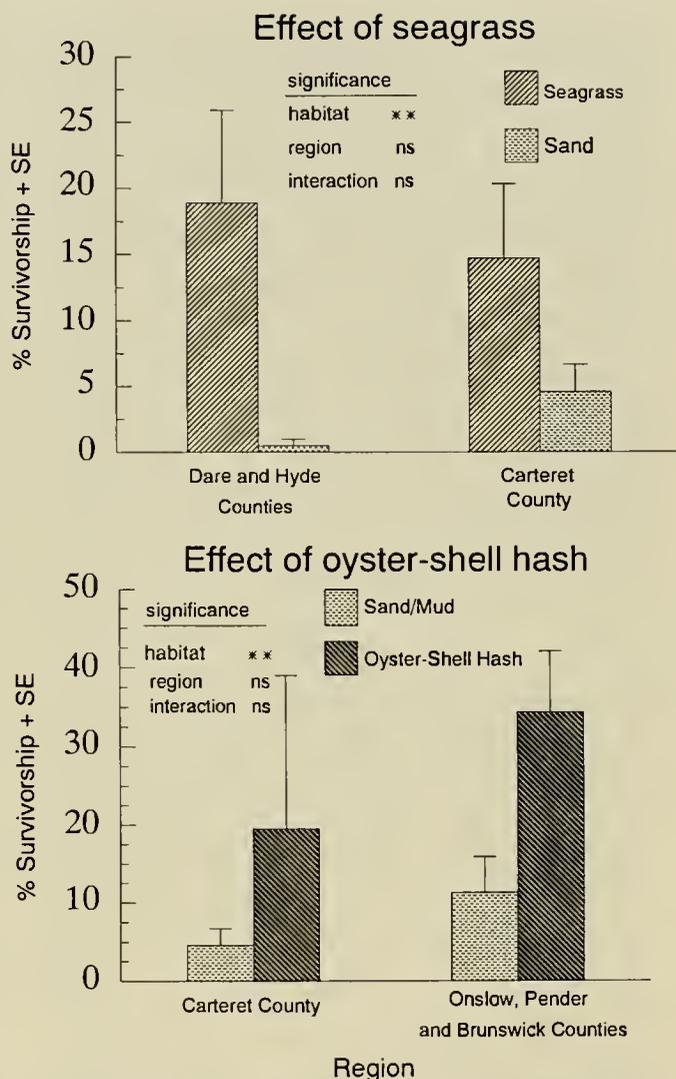


**Figure 2.** The effects of varying planting season (late summer vs. late fall/winter) on the survivorship of seed clams (*Mercenaria mercenaria*) from planting at 14–22 mm length until about 12.5% reach legally harvestable length of about 45-mm in each of 2 habitat types, seagrass and unvegetated sand. Wilcoxon signed ranks tests revealed significant effects of season at  $p < 0.05$  in sand but not in seagrass.

fall/winter plantings. Average growth, however, was greater for surviving clams from the late summer planting (1.4 vs. 0.7 cm increase in length) that had the benefit of 2.5 more months to grow.

The bottom habitat into which the seed clams were placed appeared to have a large influence on the survival of these clams to marketable size (Fig. 3). A 2-factor analysis of variance (ANOVA) (Winer 1971) comparing estimated percent survivorship of clams between regions (northern region of Dare and Hyde Counties vs. central region of Carteret County) and between habitats (unvegetated sand vs. seagrass) revealed no significant effect of region and no significant interaction but did show a highly significant difference between habitats ( $p < 0.01$ ). This test was conducted on untransformed percentages because Cochran's test (Winer 1971) revealed no significant heterogeneity of variances at an alpha of 0.05 and arcsin transformation failed to homogenize variances further (Underwood 1981). Qualitative conclusions were unchanged when analysis was conducted on arcsin-transformed proportions.

Estimated average survivorship of seed clams increased from 5% in sand to 12% in seagrass (Fig. 3). An analogous 2-factor ANOVA comparing estimated percent survivorship of clams between the central region (Carteret County) and the southern region (Onslow, Pender, and Brunswick Counties) and between habitats (unvegetated sand or sand/mud vs. oyster shell hash) also revealed a highly significant ( $p < 0.01$ ) effect of habitat but no regional difference and no interaction between region and habitat. Again, Cochran's test revealed that the percentage data did not require transformation and that neither homogeneity of variances nor the qualitative ANOVA result was altered by use of arcsin transformation. Estimated average survivorship of seed clams from planting to marketable size increased from 5–11% in unvegetated sand or sand/mud to 20–35% in oyster shell hash (Fig. 3). The average survivorship of 20% observed for the shell hash habitat in the central region is almost certainly an underestimate because it includes one replicate plot with no survivors and a greatly disturbed bottom, indicative of intense recent clamming. The other replicate plot in oyster shell hash produced an estimated 40% average sur-



**Figure 3.** The effects of bottom habitat type (seagrass vs. unvegetated sand in the top graph, and oyster-shell hash vs. sand/mud in the bottom graph) on the survivorship of seed clams (*Mercenaria mercenaria*) from planting at 14–22 mm length until about 12.5% reach legally harvestable length of about 45-mm. Two-factor ANOVAs demonstrated no effect of region and no interaction between region and habitat type in either experiment but significant effects of habitat type at  $p < 0.01$  in each case.

vival of seed clams, consistent with the 35% exhibited by this habitat on average in the southern region.

## DISCUSSION

The estimated survivorships of seed hard clams from planting to initial market size recorded in our study are astoundingly high, given that no protective measures to prevent crab predation were employed and given that previous studies of the fate of small, densely packed seed clams without predator protection in southeastern states have demonstrated massive losses to predators in very short periods of time (e.g., Menzel 1971, Gibbons and Castagna 1985, Peterson 1990). Earlier tests of the feasibility of various methods to protect seed clams against predators in aquaculture settings have shown that use of shell hash or gravel greatly reduces the losses to predators (Castagna and Kraeuter 1977), so

the enhanced survivorship of seed clams in oyster-shell hash in our study is not surprising. Furthermore, several studies have demonstrated the role of seagrass roots and rhizomes in inhibiting burrowing predators on buried invertebrates (Peterson 1982, Summerson and Peterson 1984), so the suggestion in our results of higher seed clam survivorship in seagrass habitat is consistent with previous reports. Nevertheless, what is surprising and encouraging is the generally high level of estimated survivorship recorded in our study, with averages ranging up to 35% survival from planting to market for seed planted in oyster-shell hash in the southern region of North Carolina.

This high level of survivorship was produced by a combination of all the measures taken to minimize loss to predators, namely choice of large seed size, late fall/winter planting season, low planting density, and appropriate habitats and sites within habitats. Feeding choice experiments with blue crabs as predators and *M. mercenaria* of varying sizes as prey have demonstrated that a substantial reduction in risk of predation occurs right around the size of 20–25 mm in shell length (Arnold 1984, Walker 1984, Peterson 1990). By planting extremely large seed clams, mostly in the size range of 16–22 mm in length, we successfully avoided exposing the seed clams to the highest risk from crab predation. Our choice of a late fall to early winter planting season follows the recommendations of Eldridge et al. (1979) and Peterson (1990) for the southeast: both of these papers urge planting of seed clams after water temperatures decline and blue crabs enter winter dormancy. Hard clams, especially in the smaller seed clam sizes, continue to show rapid winter growth in the southeast (Peterson and Fegley 1986), so this choice of late fall/winter planting season implies that vulnerability to crabs will be even further reduced by growth to even larger sizes by spring when the crabs again pose a threat (Peterson 1990). Our test of the influence of planting season on survivorship of seed clams revealed that this choice of a late fall to winter planting season did indeed contribute to enhancing survivorship in our study (Fig. 2).

The choice of low planting density of one clam per m<sup>2</sup> also is a likely important contributor to high survivorship of seed clams in our experimental trials. Although we were unsuccessful in testing explicitly the effect of planting density on seed clam survivorship, experimental evidence presented in Eggleston et al. (1992) on how blue crabs prey upon bivalves in a density-dependent fashion and results of manipulating planting density of seed clams in North Carolina (Summerson et al. 1995) provide support for the conclusion that the low planting density used in our trials was extremely important to the survivorship of the seed clams. Such low planting densities are usually not feasible for private aquaculture operations where leased or deeded bottom area is limited and where costs of other predator protection methods and of harvest increase directly with area. However, for the purposes of stock replenishment on public bottom, low-density planting of seed clams is a viable method of reducing losses to predators and maximizing returns. To our knowledge, this is the first study in the field that has evaluated the success of this concept.

The choice of appropriate bottom habitats and of sites within habitat makes an additional large contribution to the success of seed clam planting in our study. It seems clear from our explicit tests of habitat effects that the use of a bottom type characterized by abundant shell hash serves to enhance survivorship of seed clams (Fig. 3). This result follows what might have been expected from our previous knowledge of how addition of shell hash or gravel to aquaculture plots reduces the losses of seed clams to predators (Castagna and Krauter 1977, Gibbons and Castagna

1985). Thus, planting onto shell bottom takes advantage of the natural protection offered by shell fragments. However, one additional aspect of siting is involved in our study. We took advantage of traditional knowledge from fishermen to select specific localities that were known historically to have produced abundant hard clams. Numerous field studies have demonstrated tremendous variability on several spatial scales in ecological processes among replicate sites of what appears to be identical habitat type (e.g., Caffey 1985, Peterson and Beal 1989). Consequently, site-specific information is extremely important to the success of any culture operation. Only rarely is the extensive traditional knowledge of fishermen adequately utilized in western science or in application of science for restoration (Johannes 1976), but we endeavored in our project to overcome the vagaries of natural site variability by exploiting traditional knowledge of which sites were likely to sustain the best survivorship of our seed clams.

As one means of evaluating the success of a restocking program based upon results of our trials, we calculated the simple costs of seeding and the returns expected from the commercial harvest of the survivors (Table 3). We purchased the large seed clams at a price of \$0.028 per clam, a price that is close to the actual costs of producing the clams in a hatchery and may be difficult to duplicate. Nevertheless, the quoted price for 16–25 mm seed clams from ARC (Aquaculture Research Corporation, Den-

TABLE 3.  
Economic costs and benefits of hard clam restocking of public bottom, based upon results of North Carolina experiments.<sup>1</sup>

Parameter	Value
Seed clam (14–22 mm) cost in this project <sup>a</sup> [A]	\$0.028
Planting labor and fuel costs per clam planted [B]	\$0.002
Total costs per clam planted <sup>b</sup> [C = A + B]	\$0.030
Average observed survivorship to marketable size in oyster-shell habitat [D]	35%
Range in dockside prices paid to commercial fishermen per littleneck clam [E]	\$0.12–0.20
Return in dockside value of harvested clams per dollar invested in planting <sup>c</sup> [E/(C/D)]	\$1.40–2.33
Duration of time from planting until harvest and realization of return on investment <sup>d</sup>	14–24 months
Economic multiplier for seafood products <sup>e</sup>	1.5–2.0

<sup>a</sup> This cost may decrease or increase if seed clams are provided through construction and operation of a public-owned hatchery.

<sup>b</sup> Costs assume prior availability of a small boat for planting and do not amortize that investment.

<sup>c</sup> This return is calculated on the assumption that those clams reaching marketable size are all harvested by the commercial fishery as littlenecks (the smallest, most valuable size class).

<sup>d</sup> We know that 12.5% of surviving clams had reached legally marketable size (2.5 inch thickness) after 14 months and from growth data in Peterson and Beal (1989) estimate that all will pass this size after passage of another 10 months.

<sup>e</sup> The economic multiplier represents an estimate of the incremental economic benefits generated downstream of the purchase of seafood by commercial dealers.

<sup>1</sup> This simple analysis fails to incorporate the following additional benefits: (1) the economic benefits of supporting hatchery production of seed clams; (2) the benefits of spawn release by maturing seed clams that help repopulate public bottom; (3) the economic benefits to recreational clam fisheries, including indirect effects of enhancing tourism, retirement, and land values as well as effects of purchase of boats, gear, and other recreational equipment and supplies.

nis, MA) for 1993–1995 has been \$0.035 per clam, only slightly higher. The cost of labor to plant seed clams in a fashion appropriate for mass planting, not the careful placement at fixed positions required for our research evaluation, is \$0.002 per clam. We ultimately used a plastic jai alai scoop for scattering the clams at about 1 m distances in the mass planting and assume a wage of \$7.50 per hour. We make no addition for equipment purchase or depreciation, on the assumption that existing boats can be deployed for this activity, but we did add the costs of fuel to the planting costs. We assume that choice of the best habitat for survival and best sites within that habitat would predictably yield a clam survivorship of 35% to marketable size and that all survivors will be harvested in the commercial fishery. At a dockside price of \$0.12–0.20 per clam (varying with size and market), this reseed-ing of public shell bottom would return after about 14–24 months \$1.40 to \$2.33 for every dollar invested, under the simplified assumptions made for this analysis. This calculation would normally be augmented by an economic multiplier, typically around 1.5 to 2 for raw seafood products, to reflect the added economic value of the effect of the seafood processing, handling, marketing, etc., as the product moves downstream through the system.

This simple analysis of the economic benefits of a potential hard clam seeding program on public bottom using the most successful methods developed from our study ignores at least 3 other important economic and social benefits of such a program. First, it does not include the economic benefits of the actual purchase of the seed clams themselves and the hatchery jobs generated by that purchase. Second, it merely assesses the benefits of direct returns on the investment to the fishermen who harvested the clams. Before they are harvested, the hard clams planted as seed in such a program will spawn at least once and thereby contribute to repop-

ulation of other areas of public bottom. There is reason to argue that some shellfish populations may become recruitment limited as adult populations are depleted (Peterson and Summerson 1992), so this addition of clam larvae from spawning seed clams may make an important contribution to hard clam stocks. Third, this simple analysis assumes that the surviving clams enter the commercial fishery when in fact recreational clammers would recover some share of them. The value of enhancing the ecosystem services to the broader public and of improving the general quality and attractiveness of coastal life in the region would be difficult to quantify but is probably high, affecting tourism, retirement, and coastal land valuation. In addition, stimulation of recreational fishing opportunities for clammers itself has indirect economic benefits generated by the purchases of boats, equipment, and supplies, not taken into account in our simple calculations. Thus, the outcome of our test trials combining the effects of use of large seed clams of 14–22 mm, a late fall/winter planting season, sparse planting density of one clam per m<sup>2</sup>, and appropriate choice of shelly bottom habitat in traditionally productive sites provides encouragement for the establishment of reseed-ing programs for restocking public bottom with hard clams.

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## EARLY GAMETOGENESIS AND SPAWNING IN “JUVENILE” ATLANTIC SURFLCLAMS, *SPISULA SOLIDISSIMA* (DILLWYN, 1819)

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**ABSTRACT** The surfclam, *Spisula solidissima*, was thought to attain partial sexual maturity at 1+ years and full sexual maturity at 2+ years. Small surfclams (shell length = 7.0–10.1 mm), 3 months old or less, were collected at a shallow (15 m depth) shelf station on Beach Haven Ridge off Tuckerton, NJ on October 1, 1993. They spawned spontaneously when subjected to a temperature increase in the laboratory, and produced larvae, which subsequently died at the D-stage. Samples of small surfclams from the same station in late October and mid-November were fixed and sectioned, and individuals were classified as to sex and the stage of development of the gonads. In October, 39% of the individuals (n = 89) had ripe or ripening gonad material and the sex ratio of males to females was approximately even. Shell lengths of the smallest reproductive males and females were 3.4 mm and 5.1 mm respectively. In November, 36% of the individuals (n = 98) had ripe or ripening gonads, and there were more males (64%) than females (36%). The smallest male and female clams had shell lengths of 5.4 and 5.6 mm respectively. In October, 34% of females and 5% of males were classified as partially or fully spent, while in November 16% of females and 39% of males were classified as partially or fully spent. Thus, surfclams may reach sexual maturity and spawn within 3 months of settlement.

**KEY WORDS:** *Spisula solidissima*, gametogenesis, early spawning, mactrid, surfclam, gonad ripeness

### INTRODUCTION

*Spisula solidissima* (Dillwyn, 1817), the Atlantic surfclam, is a large bivalve ( $L_{\infty}$  = 226 mm) found in depths up to 40 m on the continental shelf from the Gulf of St. Lawrence, Canada to Cape Hatteras, NC (Merrill and Ropes 1969, Jones 1981). It is a commercially important species characterized by relatively infrequent recruitment (Murawski and Serchuk 1989, Weinberg 1993). Various hypotheses have been advanced to account for the absence of recruitment (Haskin et al. 1979, Swanson and Sindermann 1979, Garlo 1982, MacKenzie et al. 1985, Murawski and Serchuk 1989, Weinberg 1993), but no one has suggested that this results from a failure of adult surfclams to spawn each year.

Previous work has shown that surfclams can produce gametes at 1+ years of age (when the mean length is 39 mm), but that the first important spawning takes place at 2+ years when the mean length is about 67 mm (Belding 1910, Ropes 1979). Surfclams from Prince Edward Island, Canada have been reported to reach sexual maturity only after 4 years at a length of 80 to 95 mm (Sephton and Bryan 1990). Length at age varies markedly, both regionally and site-specifically (Wagner 1984, Cerrato and Keith 1992). For example, 1+ year old surfclams at 9 stations on the northern shore of Long Island varied in mean length from about 29 to 56 mm (Cerrato and Keith 1992).

As part of an ongoing, multidisciplinary study of surfclam settlement and recruitment at a 15-m shelf site on Beach Haven Ridge off Tuckerton, NJ, benthic core samples were collected every two weeks from the end of June to late December, 1993 (Grassle et al., unpubl.). A fortuitous observation of spontaneous spawning of juvenile surfclams in response to a temperature increase on October 6, 1993, led us to resample the same surfclam population in late October and mid-November for evidence of gametogenesis and spawning.

### METHODS

#### *Spawning*

Juvenile surfclams, *S. solidissima*, were collected on October 1, 1993 from Station 8 at the Rutgers University Long-Term Ecosystem Observatory at 15 m depth off the New Jersey coast (LEO-15). The clams were held at a constant temperature of 10°C or 20°C. On October 6, the clams (shell lengths of 1 to 13.3 mm) were exposed to room temperature seawater (22.5°C), and began a contagious spawning in the laboratory. We isolated spawning individuals in dishes filled with 1 µm-filtered seawater to determine their sex. A total of 21 clams were isolated, of which 6 continued to spawn after isolation. A dilute sperm suspension was added to the non-spawning individuals, and 5 more individuals spawned during the next hour.

A second attempt was made to obtain viable gametes and healthy larvae from small surfclams collected on October 25. These clams were maintained at 14°C (approximate temperature at collection), aerated, and fed *Isochrysis galbana* daily until they were strip-spawned on October 29. In females, oocytes were released into filtered seawater. In males, sperm samples were kept “dry” until used in fertilization. A total of 58 individuals were examined and 13 males and 8 females were identified as having mature sperm and oocytes.

Several batches of fertilized oocytes from both spawnings were placed at 20°C. The small volume cultures were not aerated but were fed phytoplankton daily and seawater was changed every second day.

#### *Field Collections for Histological Studies*

*S. solidissima* were collected at LEO-15 from Station 9 on October 25, 1993 and from Station 8 on November 16, 1993. They were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 24 hr, and then placed in increasing concentrations of ethyl alcohol up to 70%, until sectioned.

Prior to sectioning, clam samples were divided into 3 size

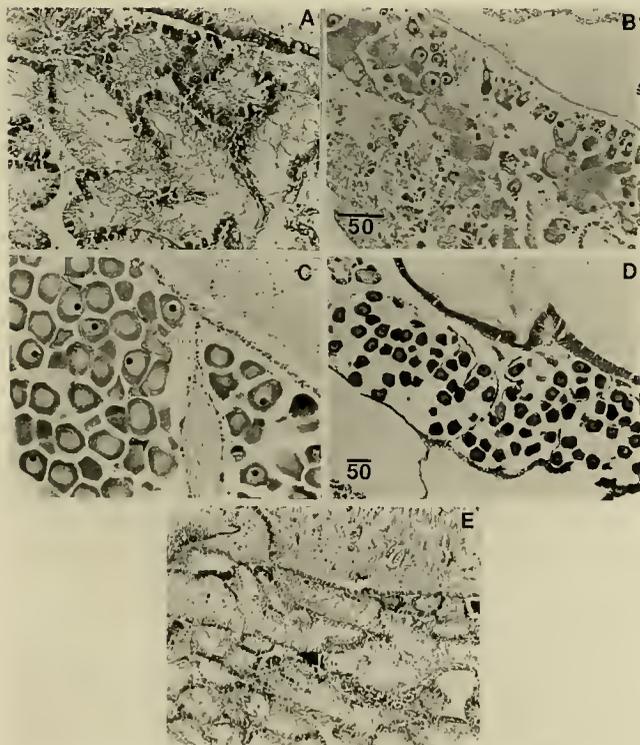
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classes based on shell length (0–5 mm, 5.1–10 mm, 10.1–15 mm). Clams were then placed in a decalcifying solution comprised of 50 mL 90% formic acid, 40 mL concentrated HCl, 410 mL distilled water until the shell was dissolved (R. Hillman, Batelle Laboratories, pers. comm.). The clams were then processed according to standard histological techniques. A total of 101 clams were sectioned from the October 25 collection and 115 from the November 16 collection. Slides were stained with Groat/Weigert Hematoxylin stain, Acid Fuchsin counterstain, Orange G and Chromotrope, followed by Fast Green/Aniline Blue staining (R. Barber, Haskin Shellfish Laboratory, pers. comm.).

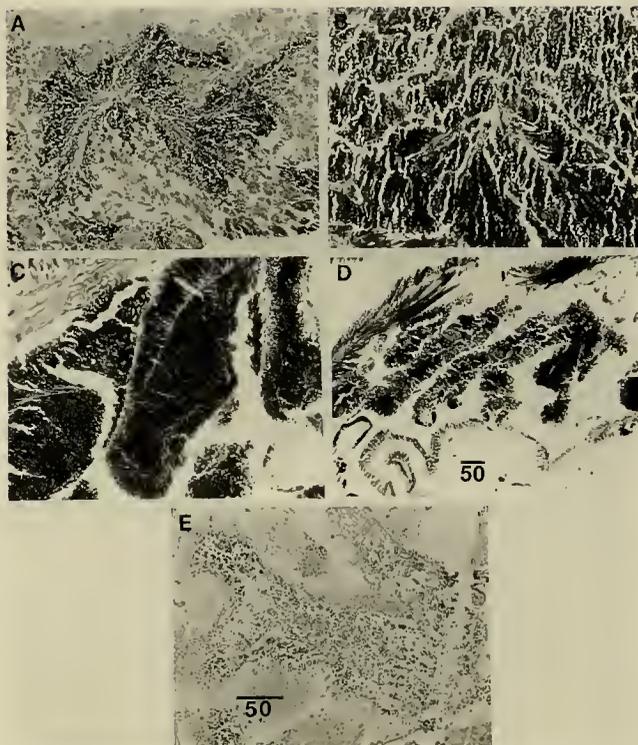
Only sections in which the visceral mass was intact and the follicles could be seen were used to determine gonad presence (89 of 101 clams in the October sample and 101 of 115 in the November sample). Individual clam sections with gonad were sexed and classified according to one of the following five ripeness classifications (based upon Ropes 1968 and modified slightly to apply to small *S. solidissima*).

### 1. Early Active Phase

In females, the oogonia were at the periphery of the follicles (Fig. 1A). Round nuclei had basophilic nucleoli and the cytoplasm was irregular in shape. Oocytes were attached to the basement membrane by a stalk. In males, the darkly stained spermatogonia



**Figure 1.** Photomicrographs of the gonadal stages of the female surfclam, *S. solidissima*. A) Early Active female (200 $\times$ ) collected in November 1993 in the 10.1–15.0 mm length size class; B) Late Active female (200 $\times$ ) collected in November 1993 in the 5.1–10.0 mm length size class; C) Ripe female (200 $\times$ ) collected in November 1993 in the 10.1–15.0 mm length size class; D) Ripe/Partially Spent female (100 $\times$ ) collected in October 1993 in the 5.1–10.0 mm length size class; E) Spent female (100 $\times$ ) collected in November 1993 in the 10.1–15.0 mm length size class.



**Figure 2.** Photomicrographs of the gonadal stages of the male surfclam, *Spisula solidissima*. A) Early Active male (200 $\times$ ) collected in October 1993 in the 0–5.0 mm length size class; B) Late Active male (100 $\times$ ) collected in October 1993 in the 5.1–10.0 mm length size class; C) Ripe male (200 $\times$ ) collected in October 1993 in the 5.1–10.0 mm length size class; D) Ripe/Partially Spent male (100 $\times$ ) collected in October 1993 in the 5.1–10.0 mm length size class; E) Spent male (200 $\times$ ) collected in November 1993 in the 10.1–15.0 mm length size class.

were in the follicular walls (Fig. 2A). Their large nuclei were round and surrounded by thin, irregularly shaped cytoplasm.

### 2. Late Active Phase

In females, oocytes were large, and often round, with conspicuous nuclei (Fig. 1B). Some oocytes were free in the lumina and others were attached to the follicle wall. In males, all spermatogenic stages were seen and the different sizes of the stages could be distinguished (Fig. 2B). Secondary spermatocytes (largest cells) had dark basophilic nuclei and irregular cytoplasm. The spermatids were half the size of the secondary spermatocytes and formed dense masses. Sperm formed weak columns in the follicle.

### 3. Ripe Phase

In females, oocytes were free in the follicle (Fig. 1C). They appeared similar to oocytes in the previous stage, except that a prominent amphinucleus consisting of an almost transparent, granular nucleolus was present. In male clams, mature sperm were uniform in size and found in dense masses in the alveoli (Fig. 2C). In some areas the sperm formed homogeneous masses, while others appeared to have been swirled toward the center of the alveoli.

### 4. Ripe/Partially Spent Phase

This phase was intermediate between the Ripe and Spent phases and was often difficult to classify. In female clams, some follicles appeared to contain a few ripe oocytes free in the lumina,

while others were empty (Fig. 1D). The visceral mass of the clam was very distended. In males, many of the spermatozoa were in the centers of the alveoli, but in reduced numbers compared with the Ripe phase (Fig. 2D).

### 5. Spent Phase

In females, the lumina of the alveoli were distinctly visible and showed few to no ripe oocytes (Fig. 1E). Alveoli were loosely arranged with mesenchyme and hemocytes sometimes visible. In males, very few of the lumina were open and a few mature sperm were still visible (Fig. 2E).

## RESULTS

### Spawning

Six of the 21 clams spawning on October 6 were males ranging in length from 7.4 to 10.1 mm, and 5 were females 7.0 to 9.8 mm in length. Cell division proceeded normally with D-stage larvae after 24 hr. After 72 hr there were many dead D-stage larvae without food in the gut. Larvae from strip spawned clams developed to the D-stage after 72 hr. The larvae appeared to be feeding on the phytoplankton cells, but no larvae were seen to develop past the D-stage.

### Field-Collections for Histological Studies

#### October 25, 1993

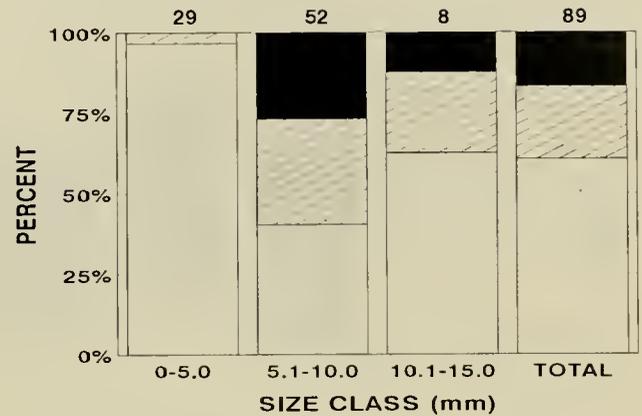
In this sample, 39.3% of all clams successfully sectioned contained ripe or ripening gonad material; 42.9% were females and 57.1% males (Fig. 3A). The smallest male clam that showed evidence of spermatogenesis was 3.4 mm in length, and the smallest classified as ripe was 5.1 mm long. The smallest female that showed evidence of oogenesis (5.1 mm) was also the smallest one classified as ripe. When the smallest, nongametogenic clams were removed from consideration (those smaller than the smallest gametogenic clam), the percent of gametogenic clams in the sample increased to 56.7%, and the male:female ratio remained essentially unchanged (55.9% males:44.1% females).

When the clams in this sample were divided into 5 mm size classes, almost 60% of the 5.1–10.0 mm (II) clams were gametogenic compared to 38% in the 10.1–15.0 mm size class (III) (Fig. 3A). Only one individual in the smallest size class (0–5.0 mm) showed evidence of gametogenesis, and this was a male. For only the gametogenic clams, in the second size class (5.1–10.0 mm), the male:female sex ratio was close to 1:1 while size class III was predominantly male (Fig. 3A).

The majority of the gametogenic clams in the October 25 sample were classified as Ripe (Fig. 4A). Most of the females were considered to be either Ripe (67%), or in the Ripe/Partially Spent (27%) phase (Fig. 4B). In contrast, most of the males tended to be in the Late Active (30%) and Ripe (65%) stages, with few individuals appearing to be Partially Spent (5%) (Fig. 4C).

Within size classes II and III, the predominant classification was Ripe (Fig. 4A). Many of the female clams in size class II were Ripe, but a total of 36% were classified as Ripe/Partially Spent or Spent (Fig. 4B). The one female in size class III was Ripe. A few males in size class II appeared to have spawned, but many were either Late Active or Ripe (Fig. 4C). The percent of Late Active clams was highest in size class III.

#### A OCTOBER 25, 1993



#### B NOVEMBER 16, 1993

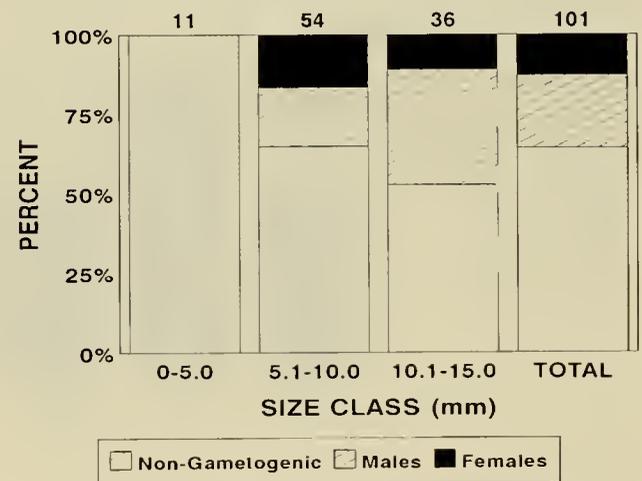


Figure 3. Percent of non-gametogenic, female and male *S. solidissima*, divided into 3 size classes based on shell length: I = 0 to 5.0 mm; II = 5.1 to 10.0 mm; III = 10.1 to 15.0 mm; TOTAL = all clams in sample regardless of size. Sample sizes are above the bars. A) all clams with interpretable histological sections in the October 25 sample, B) all clams with interpretable histological sections in the November 16 sample.

#### November 16, 1993

In this sample, 35.6% of the clams were gametogenic (Fig. 3B). The percentage of males in the sample was higher than in October, with 64% of the gametogenic clams being males. The smallest gametogenic male clam and the smallest male classified as Ripe were both 5.4 mm long. The smallest gametogenic female was a 5.6 mm clam classified as Ripe. When the smallest clams were removed from consideration, the proportion of gametogenic clams was only slightly higher, increasing to 40%. There was only a slight change in the male:female ratio when the non-gametogenic clams were excluded (67.5% males).

When the November sample was divided according to size classes, there was more gametogenic clams in size class III (47%) than in classes I (0%) and II (35%) (Fig. 3B). None of the size class I clams were gametogenic, and there also were fewer gametogenic clams in class II than in October, and more in class III than

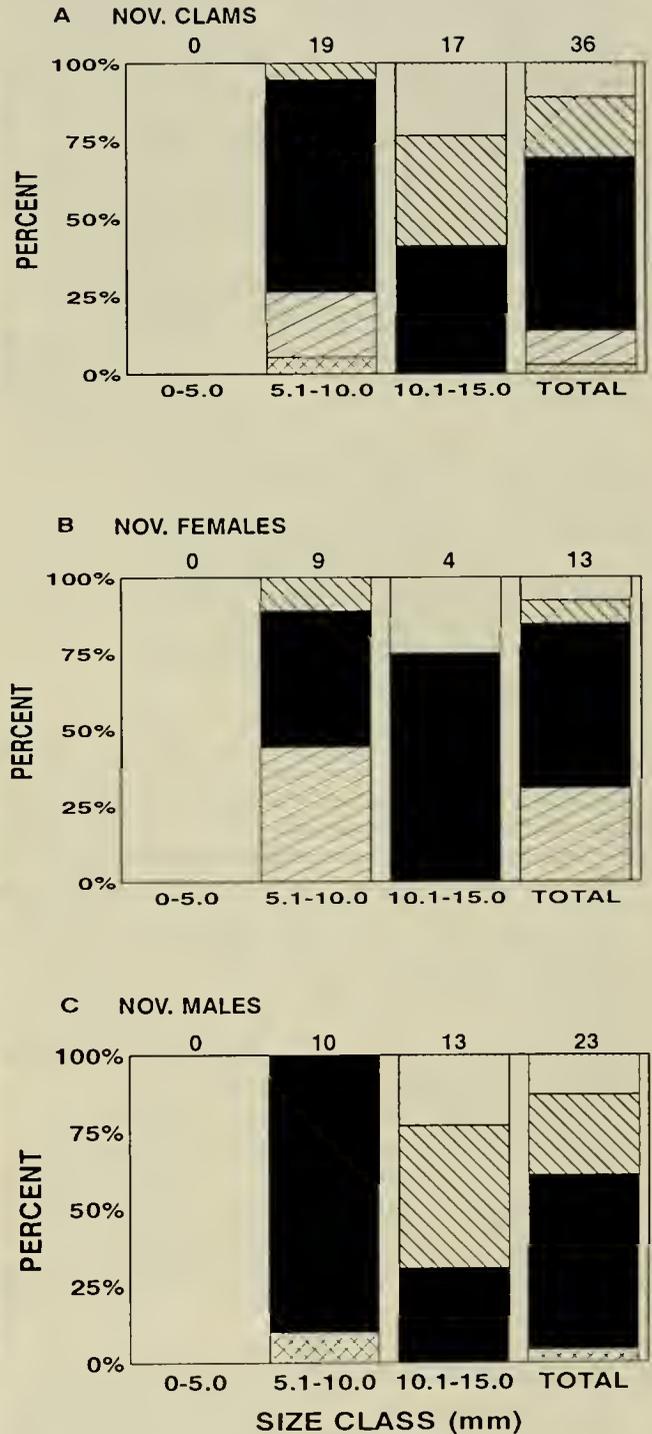
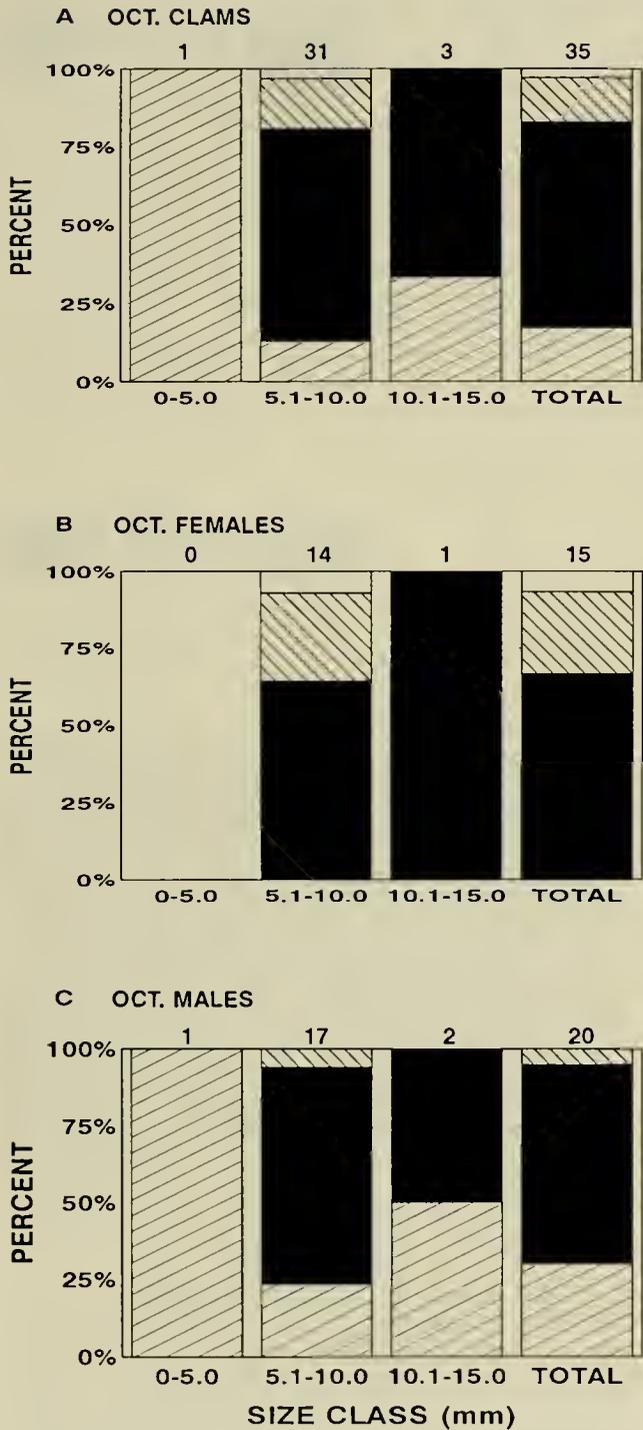


Figure 4. Percent of small *S. solidissima* in each ripeness classification collected on October 25, 1993. Samples divided into 3 size classes based on shell length: I = 0 to 5.0 mm; II = 5.1 to 10.0 mm; III = 10.1 to 15.0 mm; TOTAL = all clams in sample regardless of size. Sample sizes are above the bars. A) all surfclams combined; B) female surfclams only; C) male surfclams only.

Figure 5. Percent of small *S. solidissima* in each ripeness classification collected on November 16, 1993. Samples divided into 3 size classes based on shell length: I = 0 to 5.0 mm; II = 5.1 to 10.0 mm; III = 10.1 to 15.0 mm; TOTAL = all clams in sample regardless of size. Sample sizes are above the bars. A) all surfclams combined; B) female surfclams only; C) male surfclams only.

in October. In size class II, the sex ratio of males:females in the gametogenic clams was almost 1:1. The third size class was predominantly male, with three times as many male clams as female (Fig. 3B).

A number of the class II clams were Early Active (Fig. 5A), and none were females (Fig. 5B). More clams in size class III appeared to have spawned by this sample date (Fig. 5A). Four of the females were classified as Late Active in size class II (Fig. 5B), with an equal number being Ripe. The majority of the females in class III were Ripe (Fig. 5B). Most of the males in class II were Ripe, with a few that could be considered Early Active (Fig. 5C). Most of the males in class III appeared to be Partially Spent or Spent.

There were more Late Active females and slightly fewer Spent clams than in the previous month (Fig. 5B). There were no Late Active male clams in November, but there were a few Early Active males (Fig. 5C). The number of Ripe/Partially Spent and Spent males was higher in November at about 39% (Fig. 5C).

## DISCUSSION

The young *S. solidissima* that were the object of this study probably settled at the LEO-15 site at the beginning of July, 1993. This contention is based on an examination of benthic core samples collected at biweekly intervals from June 29 to October 15, 1993 and monthly thereafter from 3 stations on Beach Haven Ridge. A few newly settled surfclams were found in the June 29 samples, and high densities of juveniles were found in the samples taken on July 14 (Grassle et al., unpubl.). Thus, they were a maximum of 3 months old and 7–7.4 mm long when they were first observed to spawn in the laboratory in early October. Surfclams collected in late October and mid-November showed that the smallest ripe males were 5.1 and 5.4 mm in length respectively, and that the smallest ripe females were 5.1 and 5.6 mm in length. The smallest actively gametogenic individual was a 3.4 mm Early Active male collected on October 25. These observations suggest that what are usually considered juvenile *S. solidissima*, individuals 3 to 4 months in age and  $\leq 20$  mm shell length, can actually produce viable gametes and may spawn. It is probable that the contagious spawning of surfclams in the laboratory on October 6 was the result of an acute temperature increase of 2–12°C. Only one individual that was kept at 20°C after collection and then transferred to 22.4°C water is definitely known to have spawned.

Physical conditions at the LEO-15 site are characterized by frequent upwellings of cool, offshore water during the spring and summer so that it is not unusual to have rapid decreases and then increases in bottom water temperature of 4 to 10°C in less than 24 hr. In late September, immediately preceding the collection of surfclams on October 1, sea surface temperatures dropped from 21.6°C on September 24 to 18.4°C on September 26, and rose again to 22.8°C on September 27 (Crowley and Glenn 1994). The bottom temperatures at Station 9 increased from 16 to 18°C over a 12-hr time period on October 10 prior to the second collection of surfclams on October 25 (Glenn et al. 1993). These temperature changes of 3 to 4°C at the collection site could have induced small, ripe surfclams to spawn. An examination of the proportions of males and females classified as Ripe/Partially Spent and Spent in the October and November samples suggests that a higher proportion of females than males had spawned by October 25, whereas more males than females appeared to have spawned by November 16.

At a single locality, considerable interannual variation may be found in the times at which spawning in *S. solidissima* occurs. In Prince Edward Island, Canada, spawning commenced when the temperature rose to 15°C and was completed by October (Sephton 1987). However, surfclams from Chincoteague Inlet, VA spawned from mid-May to late July in 1965, and in July and August, 1966 (Ropes 1979). Inshore surfclams from New Jersey are thought to spawn from late June to November, with most of the activity concentrated from August to October (Jones 1981). In 1993, a spawning event most likely occurred in late May or early June when the temperatures had scarcely reached above 12°C (Grassle et al., unpubl.).

Following fertilization, cell division appeared to proceed normally. The timing of the first few cleavages were well within the time ranges previously noted at similar temperatures (Ropes 1980). The larvae did not develop beyond the D-stage, although some did feed. It is possible that the cultures were unsuccessful for reasons unrelated to the source of the gametes, such as the fact that some cultures were of small volume. Failure of the larvae to develop normally under similar laboratory conditions to those previously used to successfully culture surfclam larvae raises a question about whether larvae produced by spawning of young surfclams in the field survive to settlement.

There are several examples among bivalves of species that develop a small number of gametes while they are still, in effect, juveniles. This juvenile sexuality may be a different type in a young individual than that found in the adult. One example of this phenomenon, termed "consecutive sexuality" by Coe (1943), occurs in the clam, *Mercenaria mercenaria*. Almost all individuals a few months old are male. A few of these are functional but most are ambisexual, with some going on to become functional adult males while others develop into functional females. A very few individuals become hermaphrodites as adults; even fewer show evidence of a juvenile female phase and become adult females (Loosanoff 1937). Other species with early male sexuality (e.g., *Ostrea edulis* and *Ostrea lurida*) subsequently have rapidly alternating female and male phases in individuals with ambisexual gonads (Orton 1933, Coe 1943). There was no evidence among the small surfclams of ambisexual gonads or hermaphroditism. Moreover, there were approximately equal numbers of males and females in the October samples, but males did predominate in the November 16 sample (64%).

Other bivalves in the Mactridae, albeit species with much smaller maximum sizes and shorter life spans than *S. solidissima*, also quickly reach sexual maturity at a small size. These include the estuarine species, *Rangia cuneata*, which can be sexually mature at a shell length of 14 to 20 mm in North Carolina populations (Ropes 1979), and *Mulinia lateralis*, which may be sexually mature at a length of 2.7 mm after only 9 weeks of growth (Calabrese 1969). The latter species' maximum length is seldom more than 3 cm and the life span is usually less than 2 years (Scarpa et al. 1992). The two species, *S. solidissima* and *M. lateralis*, which also share some elements of genetic similarity (Rice et al. 1993), thus show interesting similarities in the early development of sexual maturity despite their very different life histories.

The main interest of these findings of early functional sexual maturity in surfclams in coastal waters lies in their significance for the population biology of the species in the region. This is especially true since there is evidence that the lack of successful recruitment in surfclams in many years is a result of intense preda-

tion on clams <30 mm in length by several species of invertebrate predators (Franz 1977, Mackenzie et al. 1985). Early reproduction within 3 months of settlement may have evolved in response to this predation pressure.

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## BOREHOLE SITE AND PREY SIZE STEREOTYPY IN NATICID PREDATION ON *EUSPIRA (LUNATIA) HEROS* SAY AND *NEVERITA (POLINICES) DUPLICATA* SAY FROM THE SOUTHERN NEW JERSEY COAST

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**ABSTRACT** Frequency, size, and site of complete and incomplete boreholes in 225 and 736 empty unbroken shells of the naticids *Euspira (Lunatia) heros* (Say 1822) and *Neverita (Polinices) duplicata* (Say 1822), respectively, from the southern New Jersey coast were measured and precisely located on the victim. Cost-benefit curves for each naticid prey species show similar slopes for largest size class of predators (outer borehole diameter = 4.0-6.0 mm). However, size refuge for *N. duplicata* and *E. heros* prey occurred at a whorl diameter (WD) of 52 and 43 mm, respectively. Prey class preferences predicted by cost-benefit analysis are congruent with actual observed predation mortalities for each prey size category. Cost-benefit analysis also shows that *N. duplicata* has greater variability in shell thickness to internal volume ratio within each size (WD) class, a suspected contributing factor to the greater (0.3 vs. 0.1) prey effectiveness of *N. duplicata* in deterring successful predation. Highest frequencies of complete boreholes occur in the first and second smallest prey size (WD) classes for *N. duplicata*, but in the second and third (next to largest) WD classes for *E. heros*. Outer borehole diameter is correlated with WD of prey shells of each naticids species ( $r = 0.67$  and  $0.71$ ), an indication of size selective predation. Whorl sector analysis and landmark triangulation shows that both complete and most incomplete boreholes are stereotypically located 180-270 degrees counterclockwise from the aperture and nearer the umbilical region than the whorl suture. Attempts to drill the umbilical plug of *N. duplicata* invariably failed.

**KEY WORDS:** Naticids, *Neverita*, *Euspira*, predation, boreholes, cost-benefit, stereotypy

### INTRODUCTION

Naticids are important predators on the subtidal to intertidal shelly benthos of the Middle Atlantic Coast, including commercially valuable clam species, among other molluscs (Kabat 1990). Moon snails bore diagnostic countersunk holes into the shells of their prey. Taphonomic processes may obfuscate the evidence in the prey shell (Kowalewski 1993), but the parabolic cross-sectional profiles in victimized shells have facilitated numerous studies of naticid prey species, size-, valve-, and/or site-selectivity (Adegoke and Tevesz 1974, Berg and Nishenko 1975, Kitchell et al. 1981, Kitchell 1986, Kabat and Kohn 1986, Aitken and Risk 1988, Kelley 1988, 1991, Anderson 1992, Kelley and Hansen 1993, Hansen and Kelley 1995) among extant and extinct molluscan prey. Correlation between predator size and prey size, as well as prey size refuge, from naticid drilling is established in most of these investigations.

Stereotypy (site selectivity) of borehole location, however, has not been documented in all investigations. Borehole site specificity by the naticids *Euspira (Lunatia) heros* (Say 1822) and *Neverita (Polinices) duplicata* (Say 1822) was observed by Berg and Porter (1974) in molluscan prey. Kelley (1991) documented stereotypic predatory behavior in extinct populations of these 2 species as well as prey effectiveness (Kelley 1988) in thwarting conspecific predation. However, site-specificity of the borehole is not ubiquitous among naticid prey (Kabat and Kohn 1986), and it should not be assumed that extant temperate latitude populations of *E. heros* and *N. duplicata* have the same: 1, correlation between predator size and prey size; 2, size refugia; 3, stereotypic borehole site specificity; and 4, cost-benefit curves as Miocene populations (Kelley 1988). Indeed, cost-benefit curves for extant populations of Floridian *N. duplicata* in experimental studies show prey size limits (inflection points) for the largest predators [outer borehole diameter (OBD) = 5.0 mm] (Kitchell et al. 1981) which are

exceeded in collections of bored shells of *N. duplicata* from the New Jersey Coast (Dietl 1995).

The objectives of this investigation were determination of: 1, correlation between predator size and prey size; 2, borehole site-selectivity; 3, prey effectiveness of each species; and 4, reconstruction and statistical comparison of cost-benefit curves for predation by populations of *E. heros* and *N. duplicata* from the New Jersey Coast. These analyses addressed several questions. Do these similar sized, co-habiting species differ in their deterrence of successful naticid predation? Are cost-benefit curves for these two naticid prey from New Jersey similar to reconstructed curves based on conspecific ancestral fossil populations from the Chesapeake Bay area which were studied by Kelley (1991)? Are unsuccessful predation attempts a consequence of suboptimum borehole sitting on the prey shell or shell thickness? In addressing the question of site stereotypy, the investigation utilized a combination of landmark morphometrics (e.g., Laurin and David 1990, Budd et al. 1994) and triangulation methodology (Kershaw and Riding 1978, Young and Scrutton 1991) for borehole location, as well as whorl sector analyses of borehole distributions (Kelley 1991).

### METHODS AND MATERIALS

Empty and hermit crab-occupied shells of the naticid snails *N. duplicata* and *E. heros* were collected from the beach and tidal flats on the Stone Harbor spit near Hereford Inlet, Cape May County, and Longsport Beach in Great Egg Harbor, Atlantic County, NJ. Whorl diameter (WD) and apertural lip thickness (ALT) were measured with Vernier calipers to the nearest 0.05 mm on all 736 and 225 shells of *N. duplicata* and *E. heros*, respectively. Specimens with complete and incomplete boreholes (Fig. 1), i.e., parabolic cross-section outline (Fig. 1B), were culled from the collection. The OBD of the countersunk borehole (Fig. 1A) was measured to the nearest 0.05 mm using Vernier calipers.

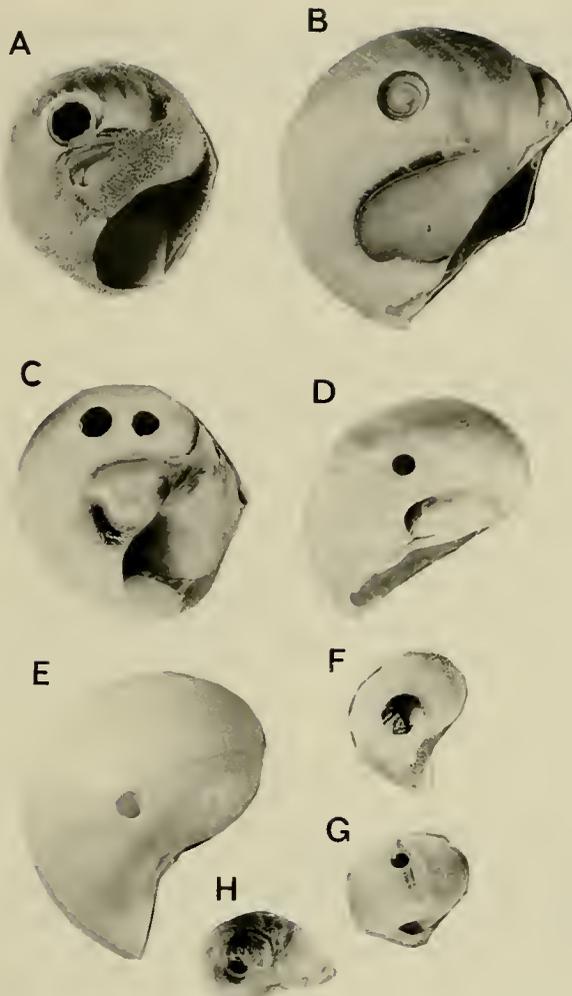


Figure 1. Specimens of *Neverita duplicata* (A–D) and *Euspira heros* (E–H) with complete and incomplete boreholes. (A) Borehole near umbilical plug. (B) Incomplete borehole. (C) Closely spaced boreholes. (D) Undersized complete borehole relative to prey size. (E) Typically located borehole. (F) Oversized borehole relative to prey size. (G) Average size borehole for prey size. (H) Apically located borehole. All specimens  $\times 1.0$ .

Kitchell et al. (1981) experimentally determined that Floridian *N. duplicata* are highly selective of their prey, and that OBD is highly correlated with the size of the predator. Accordingly, OBD was regressed on prey WD for each species to determine if predator and prey sizes were correlated for either *N. duplicata* or *E. heros* (Figs. 2 and 3). Subsequently, correlation coefficients for each species were statistically compared to ascertain if size-dependent predation differed between the 2 prey species. Furthermore, slopes ( $\beta$ ) of these regression lines were statistically compared to determine if the rate of predator size increase (OBD) relative to prey size increase (WD) differs between the naticid prey species.

Prey effectiveness (PE) was calculated as the number of incomplete drillholes divided by the total number of complete and incomplete drillholes (Kelley 1991). The higher the PE value, the more ineffective the predator is in dealing with prey. Bored specimens were sorted according to WD into 4 size class divisions, namely, class 1, <25 mm, class 2, 25–39 mm, class 3, 40–60 mm, and class 4, >60 mm (Fig. 4). Success rate of attacks by both

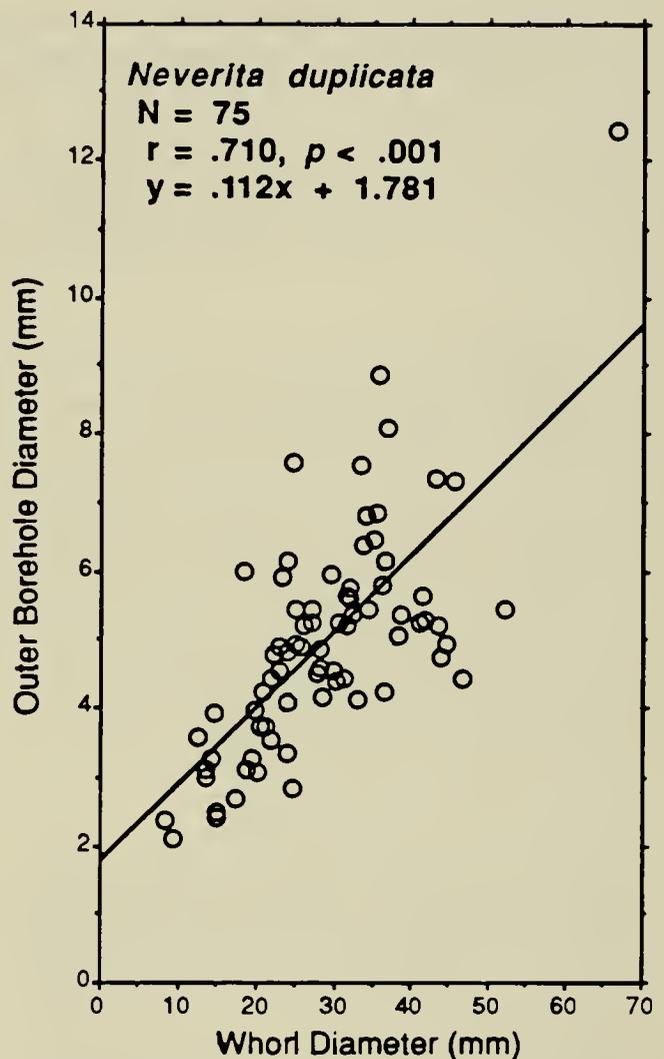


Figure 2. Relationship between predator size (indexed by outer borehole diameter) and prey size (indexed by whorl diameter) for *Neverita duplicata*.

naticid species according to size class was calculated by the percent of complete and incomplete boreholes in each size class from the total sub-sample. A Goodness of Fit test was used to determine if the frequency of complete boreholes is randomly distributed among the size classes for both *N. duplicata* and *E. heros*. Incomplete boreholes (Fig. 1B) represent interruption in the predatory event or limits of the predator in penetration of the shell of a given prey size. It is hypothesized that the mean ALT of shells with incomplete boreholes should be statistically (t-test) significantly thicker than shells with complete boreholes for each species.

The site of each drillhole was established with respect to an eight-sector grid superimposed on the shell perpendicular to the coiling axis (Fig. 5) following the procedure of Kelley (1991). Shells with incomplete body whorls were excluded because of biases introduced by missing sectors of shell. The null hypothesis that all sectors were drilled equally was tested using the Goodness of Fit test for *N. duplicata* and a Kolmogorov-Smirnov test for the smaller sample size of *E. heros*. A Kolmogorov-Smirnov two-sample test compared the distribution of complete boreholes between victimized shells of *N. duplicata* and *E. heros*.

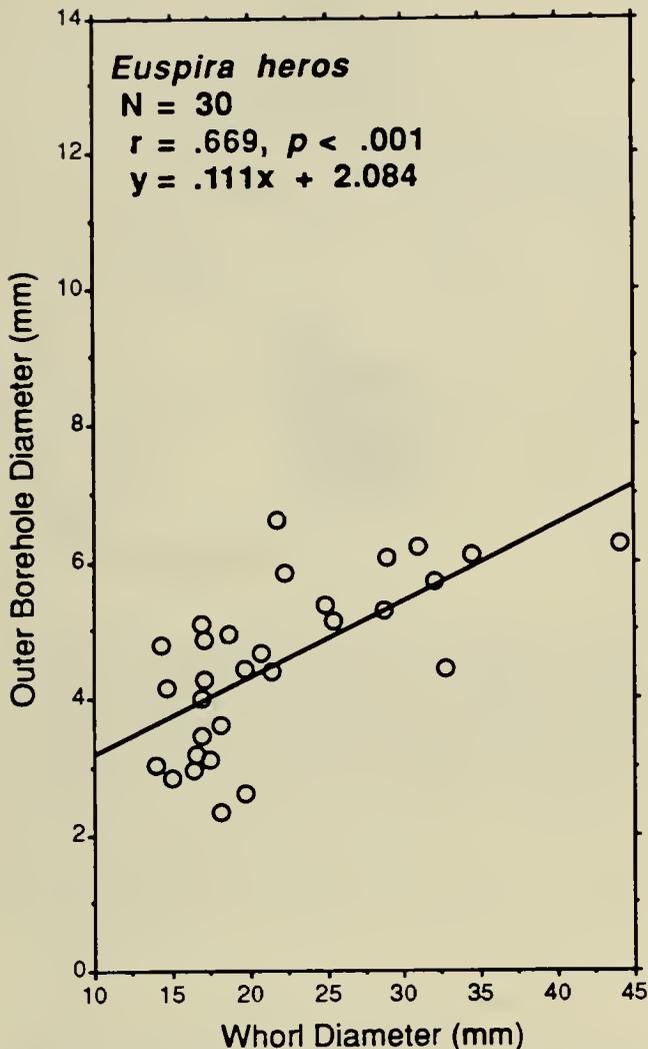


Figure 3. Relationship between predator size (indexed by outer borehole diameter) and prey size (indexed by whorl diameter) for *Euspira heros*.

For each drilling attempt (both complete and incomplete), precise borehole location was determined by measuring the distance (to the nearest 0.05 mm) from the center of the borehole to each of 3 reference points or landmarks (Budd et al. 1994), namely, 1, the umbilical margin, 2, junction of the inner apertural margin (IAM) and outer apertural lip (OAL), and 3, point of intersection of the suture between the penultimate whorl and the final whorl and the line plumbed from the apex to the junction of the IAM and OAL (Fig. 6). The distances of the borehole to each of these landmarks were summed, and the percent contribution of each line length to the total distance calculated for the specimen as per similar morphometric analyses using ternary diagrams (Kershaw and Riding 1978, Young and Scrutton 1991). Triangulated borehole centers relative to the landmarks for all complete and incomplete boreholes for each species were graphed on ternary diagrams to show any site-selectivity by the predator (Figs. 7 and 8).

Cost-benefit analysis was used to predict prey size preference for the largest predators. Kitchell et al. (1981) determined that the cost of a boring to the predator is a function of drilling time, which is controlled by shell thickness (ALT). Benefit is a function of

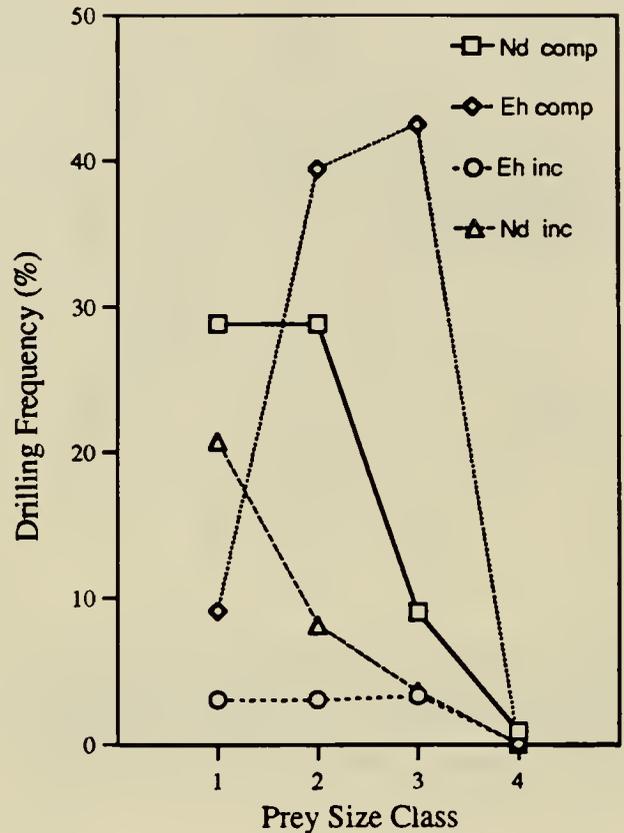


Figure 4. Percent frequency of complete and incomplete boreholes for each prey size class based on whorl diameter. Class 1 = <25 mm WD; class 2 = 25–39 mm WD; class 3 = 40–60 mm WD; and class 4 = >60 mm WD. For *N. duplicata* complete n = 75, incomplete n = 36; for *E. heros*, complete n = 30, incomplete n = 3. Null hypothesis of randomness for complete drillholes is rejected for both species ( $\chi^2 = 39.6, p < 0.001$  for *N. duplicata*;  $\chi^2 = 41.19, p < 0.001$  for *E. heros*).

prey biomass, which is highly correlated with internal volume (IV) of the shell. Potential cost-benefit curves were constructed for both *N. duplicata* and *E. heros* (Figs. 9 and 10) following the procedure of Kitchell et al. (1981) and Kelley (1991). Of 562 specimens of *N. duplicata*, a subsample of 119 specimens was randomly selected for measurement of their WD and corresponding ALT to the nearest 0.05 mm. Similarly, 113 of 225 specimens of *E. heros* were randomly selected for measurement. Internal volume (IV) was measured by filling each shell with water from a syringe. The logarithmic ratio of ALT to IV was regressed on WD for each species (Fig. 9) and their corresponding correlation coefficients statistically compared. Student's t-analysis was used to test for differences between slopes of the regression lines. If the slopes significantly differ, then the energetic yield (return) of a given sized prey item will differ between species.

Cost-benefit analysis can only be conducted for a specific sized predator (Kitchell et al. 1981). Accordingly, cost-benefit analysis was conducted for the largest size class of predators, namely an OBD of 4.0–6.0 mm. Additionally, inflection points were determined for each species to show what prey size threshold or refuge was safe from the largest predator (OBD of 4.0–6.0 mm) (Fig. 10). Prey preferences, for a predator with OBD of 4.0–6.0 mm, were predicted based on the cost-benefit curves and inflection points (Fig. 10). Theoretically, predators are most efficient when



Figure 5. Frequencies of complete boreholes in each of 8 sectors of body whorl for *N. duplicata* (shaded, innermost values) and *E. heros* (outlined, outermost values). Methodology after Kelley (1991). Null hypothesis of randomness is rejected for both species ( $\chi^2 = 124.15$ ,  $p < 0.001$  for *N. duplicata*; Kolmogorov-Smirnov Calculated  $D = 0.4$ ,  $p < 0.05$  for *E. heros*).

they bore into prey from each species which offer the lowest cost/benefit ratio in the size range of prey it can handle. Predation mortality is defined as the number of successfully drilled shells divided by the total number of shells in a given prey category (Kelley 1991). Accordingly, actual complete borehole frequencies for successive size classes of each prey species were then compared with the predicted prey preferences based on cost-benefit curves.

## RESULTS AND ANALYSIS

### Success Rate by Prey Size Class

The majority of shells of *N. duplicata* (85.2% or  $n = 627$ ) showed no sign of drilling. Only 71 specimens (9.7%) had 1 complete hole (e.g., Fig. 1A), whereas 34 shells (4.6%) had 1 incomplete hole (e.g., Fig. 1B), and 1 shell (0.1%) exhibited 1 complete and 1 incomplete hole. One shell had 1 complete and 2 incomplete boreholes (0.1%). Two shells (0.3%) had 2 complete boreholes (Fig. 1C). Out of 225 shells of *E. heros*, 192 (85.3%) shells had no sign of drilling, whereas 29 (12.9%) shells had 1 complete borehole (e.g., Fig. 1E). Three shells (1.3%) had 1 incomplete hole, and 1 shell (0.4%) had 2 complete boreholes.

Naticids successfully attacked *N. duplicata* prey in the <25 and 25–39 mm size ranges (class 1 = 28.8%; class 2 = 28.8%) at a higher frequency than larger prey (class 3 = 9.0% and class 4 = 0.9%) (Fig. 4). Size class 1 hosted 20.7% of incomplete drillholes in *N. duplicata*, whereas size classes 2 to 4 contained the remaining 11.7% of incomplete drillholes (Fig. 4). For *E. heros*, size classes 2 (39.4%) and 3 (42.4%) host the majority of complete drillholes compared with *N. duplicata* (Fig. 4). Size class 3 host 9.1% of complete boreholes, whereas class 4 showed no boreholes (Fig. 4). Size classes 1 to 3 combined contained the remaining

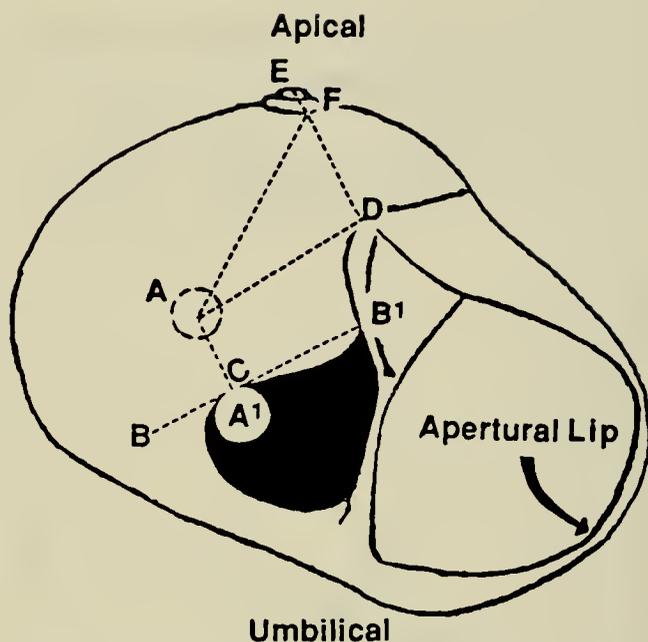


Figure 6. Shell landmarks used in triangulated drillhole location. AD—line connecting center of borehole with the junction of the inner apertural margin (IAM) and outer apertural lip (OAL); AA<sup>1</sup>—line connecting center of borehole with umbilical region perpendicular to tangent (BB<sup>1</sup>); BB<sup>1</sup>—tangent to umbilicus (or umbilical plug); D—junction of IAM and OAL; ED—line connecting apex to junction of IAM and OAL; EF—point on suture between the penultimate whorl and final whorl plumbed from the apex to junction of IAM and OAL; AF—line connecting center of borehole with point on suture between penultimate and final whorls (plumbed from apex to IAL and OAL); C—point on tangent BB<sup>1</sup> perpendicular to line AA<sup>1</sup>. Triangulation based on lines AF, AD, and AA<sup>1</sup>.

9.1% of incomplete boreholes. A Goodness of Fit test of complete drillholes according to prey size class indicates a non-random distribution for *N. duplicata* ( $\chi^2 = 39.6$ ,  $p < 0.001$ ) and *E. heros* ( $\chi^2 = 41.19$ ,  $p < 0.001$ ). Larger prey size classes for both species were infrequently attacked. PE for *N. duplicata* and *E. heros* were 0.3 and 0.1, respectively, which means that predators were three times less effective preying upon *N. duplicata* than on *E. heros*.

Naticid predator size is correlated with prey size for each species. A significant correlation occurred between OBD and WD for 75 drilled specimens of *N. duplicata* ( $r = 0.71$ ,  $p < 0.001$ ) (Fig. 2) and 30 drilled specimens of *E. heros* ( $r = 0.67$ ,  $p < 0.001$ ) (Fig. 3). The  $r$ -coefficients are statistically similar which indicates similar variation in OBD relative to WD ( $z = 0.35$ ). However, slopes of the 2 regression lines are significantly different ( $t = 2.86$ ,  $DF = 101$ ,  $p < 0.01$ ), which means that the rate of increase in WD relative to the increase in OBD is different between the prey species. Thus, a similar sized predator (comparable OBD), selected larger *N. duplicata* prey on average compared to *E. heros* prey (WD), for each predator size class (Figs. 2 vs. 3). Mean ALT for specimens of *N. duplicata* was 0.60 mm and 0.25 mm for specimens with incomplete and complete boreholes, respectively. Mean ALT for specimens of *E. heros* was 0.38 mm and 0.18 mm for specimens with incomplete and complete boreholes, respectively. Mean ALT of shells with incomplete vs. complete boreholes for specimens within each species differ significantly (for *N. duplicata*,  $t = 5.064$ ,  $DF = 103$ ,  $p = 0.0001$ ; for *E. heros*,  $t = 3.256$ ,  $DF = 31$ ,  $p = 0.0027$ ).

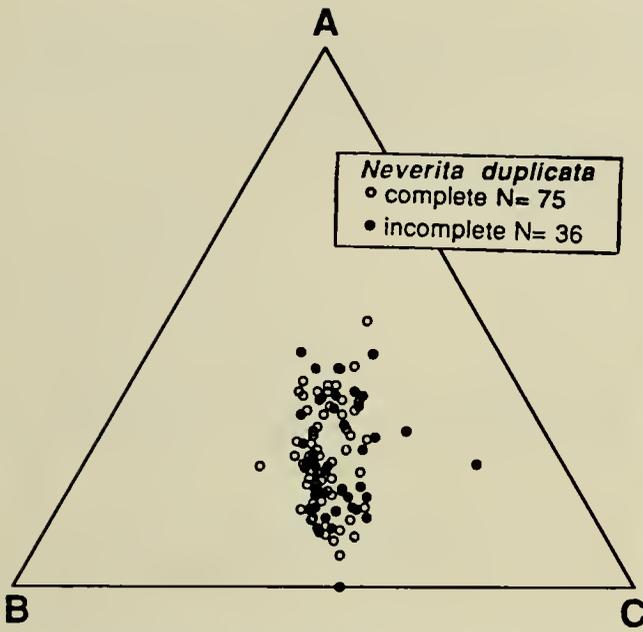


Figure 7. Ternary diagram of shell landmarks for complete (open dot) and incomplete (closed dot) boreholes for *N. duplicata*. (A) Distance from center of borehole to umbilical region. (B) Distance from center of borehole to junction of apertural lip with body whorl. (C) Distance from center of borehole to a point on the suture between the penultimate and final whorls (plumbed from apex to the IAM and OAL). See Figure 6.

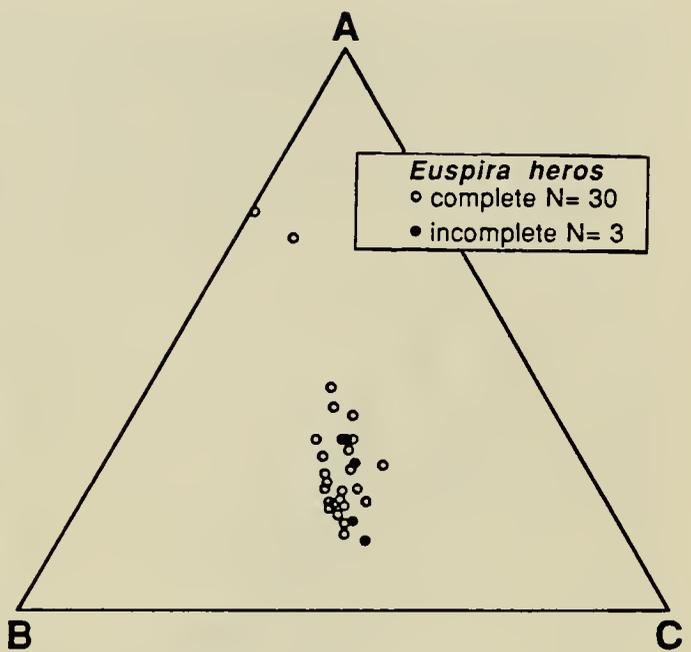


Figure 8. Ternary diagram of shell landmarks for complete (open dot) and incomplete (closed dot) boreholes for *E. heros*. (A) Distance from center of borehole to umbilical region. (B) Distance from center of borehole to junction of apertural lip with body whorl. (C) Distance from center of borehole to a point on the suture between the penultimate and final whorls (plumbed from apex to the IAM and OAL). See Figure 6.

#### Drillhole Site Selectivity

Drillhole site selectivity according to body whorl sector location occurred in both *N. duplicata* and *E. heros* (Fig. 6) (for *E. heros*,  $D = 0.4$ ,  $p < 0.05$ ; for *N. duplicata*,  $\chi^2 = 124.15$ ,  $p < .001$ ). Sixty percent and 78.67% of completed drillholes in *E. heros* and *N. duplicata*, respectively, occurred in sectors 2 and 3 (Fig. 6). A Kolmogorov-Smirnov 2-sample test indicated no significant difference in sector preference of boreholes between the 2 prey species ( $D = 0.5$ ,  $p = 0.3173$ ). Ternary diagrams of complete and incomplete borehole locations show site selective behavior for both species (Figs. 7 and 8). Additionally, this site selectivity doesn't change with size of the predator or prey as evidenced by the high correlation coefficients between each prey size (WD) and distance of borehole to each reference point in the triangulation (Table 1). Concerning *N. duplicata*, complete and incomplete drillholes overlap appreciably in "morphospace" (Fig. 7), although attempted borings nearest the umbilical plug or suture were unsuccessful. In the case of *E. heros*, the few incomplete boreholes (Fig. 8) lie within the field occupied by complete boreholes.

#### Potential Cost-Benefit Analysis

Linear regression analysis of the data used to construct the potential cost-benefit curves for the largest predatory *N. duplicata* and *E. heros* (Fig. 10) indicates the slope of the regression lines are similar ( $t = 1.43$ ,  $DF = 228$ ). Thus cost is decreasing and the benefit increasing to the predator at the same rate as size increases within either prey species. However, degree of correlation between log ALT/IV versus WD between the 2 species (Fig. 9) is statistically different ( $r = 0.47$  and  $0.69$ ,  $z = 2.52$ ,  $p < 0.01$ ). Furthermore, inflection points for a predator with OBD of 4.0–6.0

mm preying on *N. duplicata* and *E. heros* are 52 mm and 43 mm, respectively (Fig. 10). The size class (WD) of prey with the lowest cost-benefit ratio for *N. duplicata* is 37–52 mm and theoretically should be selected first by the predator (Table 2). The least preferred prey should be *E. heros* with a WD between 14–22 mm (Table 2). Observed predation mortalities are similar to those predicted by cost-benefit analysis for *N. duplicata* (Table 2). Predation on the predicted first, second, and third ranked prey of *N. duplicata* was 12.5%, 6.5%, and 4.8%, respectively (Table 2). Predation on the predicted second, third and fourth ranked prey for *E. heros* was 6.7%, 4.6%, and 4.2%, respectively (Table 2).

#### DISCUSSION

It has been hypothesized that prey size selection by naticids is indiscriminate (Stanton and Nelson 1980, Carrier and Yochelson 1968, Carrier 1981, Vermeij 1978). Presence of double complete boreholes in a shell (Fig. 1C), the newest of which may have been drilled into an already digested prey, can be adduced as support for such contentions. An alternative hypothesis to predator ineptitude is that confamilial predation results from absence of the more preferred bivalve prey species. Kitchell et al. (1981) observed experimentally that cannibalism among individuals of *Polinices duplicatus* resulted from selective predation to maximize energy gain. Accordingly, cannibalism is a normal behavior of naticids that are foraging optimally rather than a reflection of predator ineptitude or absence of bivalve prey, a conclusion in which Kelley (1991) concurred. Results of this investigation on temperate latitude populations of *N. duplicata* and *E. heros* support the conclusions of Kelley (1991) and Kitchell et al. (1981). Naticids

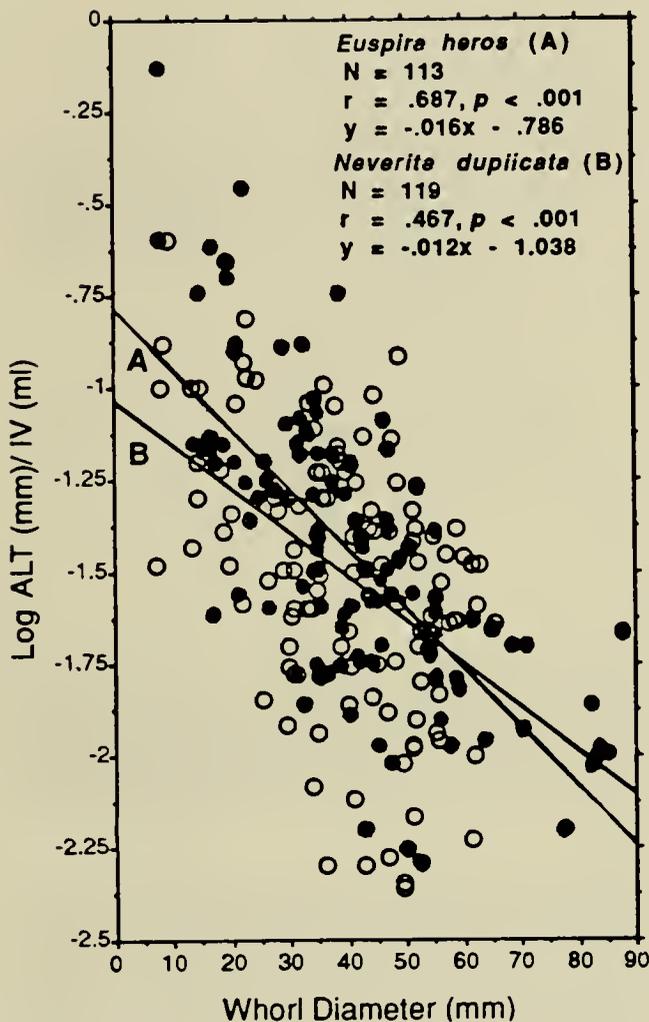


Figure 9. Regression lines used to construct potential cost-benefit curves for *E. heros* (A, closed dot) and *N. duplicata* (B, open dot). ALT = apertural lip thickness (mm), and IV = internal volume (mL). Correlation coefficients ( $r$ ) for each species are significantly different ( $z = 2.52, p < 0.01$ ).

were size selective of their moon snail prey based on correlation of predator size (OBD) with prey size (WD) (Figs. 2 and 3). Furthermore, the degree of correlation between predator and prey size has not changed significantly since the Miocene for *N. duplicata* ( $r = 0.71$  in Fig. 2 vs. 0.84 of Kelley 1991) or *E. heros* ( $r = 0.67$  in Fig. 3 vs. 0.57 of Kelley 1991).

Site specificity (Figs. 5, 7, and 8) indicates inheritance of stereotypic behavior in these 2 species that was first documented for Chesapeake Miocene populations (Kelley 1991). The same body whorl areas (Fig. 1D, E, F) with two-thirds of the boreholes (sectors 2 and 3) (Fig. 5), also hosted 88% of the boreholes in the Miocene populations (Kelley 1991). Despite nearly 20 million years of evolution of this predator-prey interaction, predatory behavior has not become more stereotypic; the number of drilled body whorl sectors of *N. duplicata* (5 of 8) and *E. heros* (6 of 8) (Fig. 5) has not decreased in comparison to Miocene populations (Kelley 1991). Based on landmark triangulation, naticids do infrequently completely penetrate atypical regions of prey, namely near the apex of *E. heros* (Fig. 1H) or near the umbilical plug of

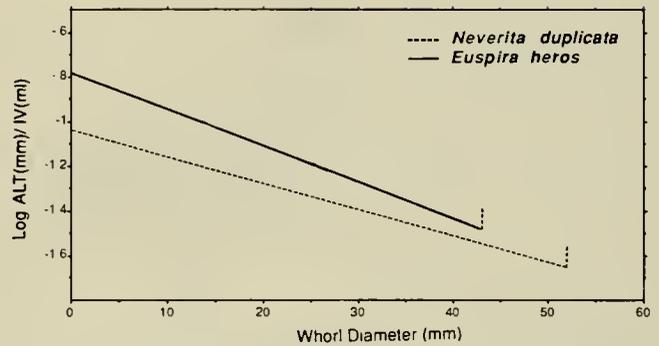


Figure 10. Potential cost-benefit curves for *E. heros* (solid line) and *N. duplicata* (dashed line). ALT = apertural lip thickness (mm) and IV = internal volume (mL). Inflection points (prey size refuge) for a predator with outer borehole diameter of 4.0–6.0 mm are 52 mm for *N. duplicata* and 43 mm for *E. heros*. For *E. heros*,  $\log \text{ALT}/\text{IV} = -0.016 \text{WD} - 0.786$ . For *N. duplicata*,  $\log \text{ALT}/\text{IV} = -0.012 \text{WD} - 1.038$ . Predicted prey rankings based on curves are for *N. duplicata* prey class (whorl diameters) 37–52 mm (1st); *N. duplicata* prey class 22–37 mm and *E. heros* prey class 31–43 mm (2nd); *N. duplicata* prey class 9–22 mm and *E. heros* prey class 22–31 mm (3rd); *E. heros* prey class 14–22 mm (4th).

*N. duplicata* (Fig. 1A). However, the few recorded attempts to penetrate the umbilical plug of *N. duplicata* failed (Fig. 7), an indication of occasional ineffective prey manipulation. Nevertheless, the clustered distribution of borehole locations on these 2 naticid prey (Figs. 6 and 7) indicates that stereotypic predatory behavior by *N. duplicata* or *E. heros* has not relaxed appreciably since the Miocene.

Stereotypic borehole siting (Fig. 1D, E) may allow the predator's proboscis to penetrate the entire shell unlike invasive points located at the apical or apertural extreme. Exceptions usually involve small prey (Fig. 1H) and/or big predators (Fig. 1A). Stereotypy of borehole location may depend as much on the prey shell morphology as concentration of prey tissue within it. Berg (1975) suggested that behavioral differences in envelopment and holding of the prey by the predator's foot influenced species-specific patterns. Relative convexity of the prey shell may be important, as evidenced by the fact that not only are certain sectors of the

TABLE 1.

Regression equations relating average distance of drilling site to prey length (WD) for 3 reference points.

Species	Ref. point	Equation	$r$	$p$
<i>N. duplicata</i>	A	$y = 0.325x - 0.541$	0.677	<0.001
	B	$y = 0.655x - 3.889$	0.718	<0.001
	C	$y = 0.599x - 2.369$	0.841	<0.001
<i>E. heros</i>	A	$y = 0.243x + 1.287$	0.646	<0.001
	B	$y = 0.274x + 2.931$	0.613	<0.001
	C	$y = 0.333x + 2.117$	0.642	<0.001

(A) Distance from center of borehole to umbilical region. (B) Distance from the center of the borehole to the junction of the inner and outer apertural lip (IAM, OAL). (C) Distance from the center of the borehole to a point on the suture between the penultimate and final whorls (plumbed from the apex to the IAM and OAL) for 75 and 30 drilled specimens of *N. duplicata* and *E. heros*, respectively.

TABLE 2.

Predicted and observed prey preferences for successive size classes of a predator with an OBD of 4.0–6.0 mm based on potential cost-benefit curves.

Predicted Prey	Observed Predation Mortality
1. <i>N. duplicata</i> 38.0–52.0 mm	10/80 = 0.125
2. <i>N. duplicata</i> 22.0–37.0 mm equivalent to <i>E. heros</i> 31.0–43.0 mm	20/310 = 0.065 2/30 = 0.067
3. <i>N. duplicata</i> 9.0–22.0 mm equivalent to <i>E. heros</i> 22.0–31.0 mm	1/21 = 0.048 3/65 = 0.046
4. <i>E. heros</i> 14.0–22.0 mm	4/95 = 0.042

Predation mortality is the number of successfully drilled shells divided by the total number of shells in a given prey category (Kelley 1991).

body whorl preferentially drilled (Fig. 5), but also certain positions along the apical-umbilical axis within those sectors (Figs. 7 and 8).

Cost-benefit analysis (Figs. 9 and 10) also shows that naticids were selective of prey size among *E. heros* and *N. duplicata*. Larger predators attacked larger prey and thereby maximized nutritional gain in return for energy spent drilling through the shell of the victim. However, the escape size is almost 10 mm larger for *N. duplicata* (Fig. 10), despite the fact that this species is significantly thicker than *E. heros*, which suggests that the added drilling time to penetrate the thicker shell is energetically compensated by the mass of meat inside. Regardless, the gain in predation on larger size naticids may be small based on the gentle slopes of the cost-benefit curves for both species (Fig. 10). Confamilial or conspecific predation does not provide the rate of increased energetic yield with increased prey length comparable to that in naticid predation on most bivalve prey, as documented by Kitchell et al. (1981). Overall predation mortalities for *N. duplicata* (10.2%) and *E. heros* (13.3%) were lower than those reported by Kelley (1991) for *N. duplicata* (14.3%) and *E. heros* (28.7%) in Chesapeake Group naticid gastropods.

Prey effectiveness in deterrence of drilling is 3 times higher for *N. duplicata* than *E. heros* (0.3 vs. 0.1). For any given WD of potential prey for *N. duplicata* there is greater variation in the ratio of ALT/IV compared with *E. heros* (Fig. 9); hence the lower correlation coefficient ( $r = 0.47$ ) for log ALT/IV regressed on prey size (WD) for *N. duplicata* relative to *E. heros* ( $r = 0.69$ ). Predators conditioned to preying on certain size classes will encounter more variation in shell thickness when attacking *N. duplicata* than *E. heros*. This increased variation in shell thickness for each prey size probably attributed to increased frequency of failure and incomplete drillholes, and thus a higher PE, for *N. duplicata*.

Also contributing to prey effectiveness is the high mobility of the prey, a fact noted by Kelley (1991). Incomplete boreholes are evidence of an interruption in the predatory event or limits of the predator in handling a given prey size. Kitchell et al. (1981) demonstrated that the probability of interruption is proportional to drilling time to penetrate the shell, which may take many hours, and hence related to prey shell thickness. Abnormally sited boreholes in the thicker area of the shell, such as near the umbilical region (Fig. 1A) suggest inability of a predator to manipulate every prey. Although a few abnormally sited boreholes are com-

plete (e.g., Fig. 1A), no attempt to drill through the umbilical plug of *N. duplicata* succeeded. Normally sited incomplete drillholes (Fig. 1B) may be evidence of interruption by external agents, but atypically sited incomplete boreholes more likely represent failure to manipulate the prey. Temporary escape and recapture following such interruptions may lead to multiple boreholes (Fig. 1C).

Alternatively, multiple, closely positioned, complete boreholes in the same shell may also indicate failure to recognize live prey. Naticids chemically sense the odors of their prey via their siphon (Kabat 1990). Yet despite this chemoreceptive ability, naticids may occasionally mistake empty or decaying flesh-filled moon snail shells for live prey. Infrequent occurrence of "reverse" countersunk boreholes, with the larger outer borehole diameter and smaller inner borehole diameter on the valve interior and exterior, respectively, on the subtropical bivalve *Chiona cancellata* indicates that moon snails occasionally mistake disarticulated clam shells for live prey (personal observation). Indeed, one specimen of *N. duplicata* shows slightly overlapping complete boreholes resembling the outline of the number 8. If the prey survived the first attempt, there is an obvious energy waste by the predator which failed to reoccupy the previous drilling site. Stereotypy of naticid predatory patterns may not be sufficiently flexible on recaptured prey (Kitchell 1986).

In the present study, the frequency of incomplete drillholes is low (Figs. 7 and 8), particularly on the thinner shelled *E. heros* ( $n = 3$ ), suggesting that confamilial or conspecific predatory behavior in naticids is efficient. This efficiency is attributable to the short time to drill through thin relative to thick shelled bivalve prey species as documented by Kitchell et al. (1981). Predatory attacks on naticid size classes below the escape size have a high probability of success. Although confamilial or conspecific predation is a preferred behavior of selective predation and maximized energy, the high mobility of naticids inhibits capture by other moon snails (Kelley 1991), which accounts for more than 85% of the collected shells of *N. duplicata* and *E. heros* from the southern New Jersey Coast lacking boreholes.

## CONCLUSIONS

Stereotypic behavior in site of borehole and size of naticid prey is documented for both *Euspira (Lunatia) heros* and *Neverita (Polinices) duplicata*. Outer borehole diameter, an index of predator size, is significantly correlated with prey whorl diameter for both species. Both species are preferentially drilled in 2 sectors of the body whorl slightly closer to the umbilical than the sutural margin of the whorl based on landmark triangulation. Incomplete boreholes on *E. heros* and the overwhelming majority of *N. duplicata* do not deviate significantly in location from the clustered sites of complete boreholes, although the few attempts to drill the umbilical plug of *N. duplicata* failed. Moon snails are three times as ineffective preying on the thicker *N. duplicata* (0.3) than *E. heros* (0.1), based on the ratio of incomplete to total attempted boreholes. Potential cost benefit curves (log of apertural lip thickness/internal shell volume vs. prey whorl diameter) are similar between the species, although the degree of correlation ( $r$  coefficients) for each species is significantly different. Prey class preferences predicted by cost-benefit analysis are congruent with actual observed predation mortalities for each prey size category. The higher frequency of incomplete boreholes in *N. duplicata* may be attributed to the greater variation in shell thickness for any given prey size (whorl diameter) relative to similar sized *E. heros*. Significantly

thicker shells of the same prey size (whorl diameter) class are less likely to be completely drilled through by same size predators. The escape size for a maximum sized predator (OBD = 4.0–6.0 mm) is 52 mm and 43 mm for *N. duplicata* and *E. heros*, respectively. A given size predator commonly selected a larger prey of *N. duplicata* compared to *E. heros* over the range of prey sizes.

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## RELATIONSHIPS BETWEEN SEAGRASS BED CHARACTERISTICS AND JUVENILE QUEEN CONCH (*STROMBUS GIGAS* LINNE) ABUNDANCE IN THE BAHAMAS

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**ABSTRACT** Juveniles of the large, commercially significant gastropod *Strombus gigas* Linne (queen conch) are aggregated year after year in specific sectors of large seagrass meadows on the Great Bahama Bank. The association of conch with particular nursery grounds was investigated at 23 stations near Lee Stocking Island, Exuma Cays, Bahamas. The stations were chosen to represent locations both within and outside the nursery sites, and matched as closely as possible in terms of depth, seagrass density, and sediment characteristics. Principal component analysis indicated that no single habitat variable was a good predictor of juvenile conch abundance. Seagrass biomass and density, sediment organics, and algal abundance were all important elements associated with conch abundance. All of these variables were generally low at stations with no long-term record of conch habitation. Seagrass is the most conspicuous feature of seagrass beds, but the variable abundance of other important food items, hydrographic differences, and differences in the supply of larvae to specific locations result in large-scale faunal distribution patterns relatively independent from seagrass biomass. Conch nursery grounds were associated with specific combinations of food production and shelter not immediately obvious within large meadows. Most seagrass beds are probably unsuitable for aggregations of juvenile queen conch; therefore, stock management programs will need to identify and concentrate on the unique nursery habitats.

**KEY WORDS:** Bahamas, macroalgae, multivariate analysis, seagrass, sediments, *Strombus gigas*

### INTRODUCTION

In the last 20 years, a large literature has accumulated on relationships between the characteristics of seagrass meadows and their faunal assemblages. Early observations of correlations between seagrass biomass or shoot count and abundance of fishes and invertebrates continue to be reported (Heck and Orth 1980, Stoner 1980a, 1983, Peterson et al. 1984, Humphries et al. 1992), and have been tested experimentally with small epibenthic invertebrates such as amphipods and molluscs (Stoner and Lewis 1985, Edgar and Robertson 1992). However, the direct relationship between seagrass quantity and faunal density appears to break down over distances of kilometers (Heck 1977, Brook 1978, Heck and Thoman 1984), over depth gradients (Jackson 1972, Mazella et al. 1989), and with distance into estuaries (Bell and Westoby 1986, Bell et al. 1988, Humphries et al. 1992) or away from open ocean (Sogard et al. 1987, Stoner et al. 1995). It is clear that seagrass beds are highly variable in attributes other than seagrass biomass or shoot count, and that many of the less obvious characteristics of the meadows have a strong impact on the large-scale distribution of fishes and invertebrates.

An important seagrass-associated invertebrate of the greater Caribbean region is the large gastropod, *Strombus gigas* Linne (queen conch), which represents one of the region's most important fisheries (Brownell and Stevely 1981). Queen conch are most numerous on island shelves and shallow banks where their macrophyte foods are abundant (Randall 1964, Weil and Laughlin 1984, Stoner et al. 1994, Stoner and Schwarte 1994). This shallow depth distribution results in high vulnerability to fishing pressure, and populations have declined precipitously over recent years (Berg and Olsen 1989, Appeldoorn 1994).

Release of hatchery-reared juveniles has been promoted as a potentially viable means of queen conch stock enhancement and rehabilitation (Berg 1976, Laughlin and Weil 1983, Davis et al. 1987, Creswell 1994). However, virtually all of the pilot release projects have resulted in very high mortality rates (Appeldoorn and Ballantine 1983, Appeldoorn 1985, Iversen et al. 1986, Coulston et al. 1987, Rathier 1987, Davis et al. 1992). Failure is probably related to various deficiencies in hatchery-reared conch (Marshall 1992, Stoner and Davis 1994) and poor site selection for releases (Stoner and Sandt 1992a, Stoner 1994).

In the Bahamas, the majority of juvenile conch inhabit seagrass meadows on the shallow banks (Robertson 1961, Iversen et al. 1987) close to deeper shelf environments where reproductive populations are most abundant (Stoner and Sandt 1992b, Stoner and Schwarte 1994). In the Exuma Cays, Bahamas, historically large aggregations of juvenile conch occur in the same sectors of large seagrass beds year after year (Stoner et al. 1994, 1995). Stoner and Waite (1990) showed that juvenile conch occupy areas of intermediate density of seagrass biomass and that this association may be partly affected by habitat choice. Transplant experiments have shown that sites outside the immediate vicinity of historically important conch nurseries did not support normal growth in juvenile conch, despite suitable depth, seagrass biomass, and sediment characteristics (Stoner and Sandt 1991, Stoner et al. 1994). This suggests that other factors may also determine habitat quality for juvenile queen conch.

In this study we analyze a large number of habitat characteristics across several long-term conch nursery areas to test the hypothesis that juvenile queen conch distributions can be predicted on the basis of environmental characteristics. Identification of these characteristics is important to the management of critical

nursery grounds for this commercially threatened species, and will assist in habitat selection for stock enhancement programs.

## METHODS

### Study Site

The Exuma Cays, a primary source of queen conch for the Bahamas fishery, comprise a 250 km long island chain that lies along the western side of Exuma Sound (Fig. 1). Most of the low, carbonate islands are less than 8 km long and separated by inlets ranging in width from 0.2 to 1.0 km. To the east of the islands (windward in prevailing conditions), a narrow shelf, usually <2 km wide, separates the islands from the deep oceanic waters of Exuma Sound. To the west of the cays is the Great Bahama Bank, a shallow carbonate platform covered with sand and seagrass, that extends from the islands 26–65 km to the Tongue of the Ocean. Depths of 1–5 m are typical on the bank and there is a tidal range of approximately 1 m. Nursery grounds for queen conch are located primarily on the bank, while most of the spawning population is on the island shelf in 10–20 m water depth (Stoner and Sandt 1992b, Stoner et al. 1994, Stoner Schwarte 1994).

Aggregations of juvenile conch near Lee Stocking Island were mapped and examined for population size between 1988 and 1992 (Stoner et al. 1994, 1995). On the basis of this earlier descriptive work, 4 nurseries were selected for analysis in this study (Fig. 1). Shark Rock and Children's Bay Cay nurseries were large and were observed in the same general location every year since 1988. Tugboat Rock and Neighbor Cay nurseries were smaller and relatively ephemeral. Although the surface area of the Tugboat Rock juvenile aggregation was large during some years, conch density tended to be low, and there was large inter-annual variation in population size. The Neighbor Cay population has rarely exceeded a few thousand individuals and disappeared entirely in 1992 and 1993, whereas Shark Rock and Children's Bay Cay populations have included between  $10^4$  and  $10^6$  juvenile conch every year.

At the 3 largest nursery sites, stations were chosen to represent locations within the long-term nursery site ("population centers") as well as locations outside the nurseries in different directions relative to the flood tidal current (Fig. 1). Stations were placed (1) upcurrent and toward the Exuma Sound from the nurseries, (2) downcurrent, and on the bank side of the nurseries, and (3) across the current from the nurseries. At Shark Rock and Children's Bay

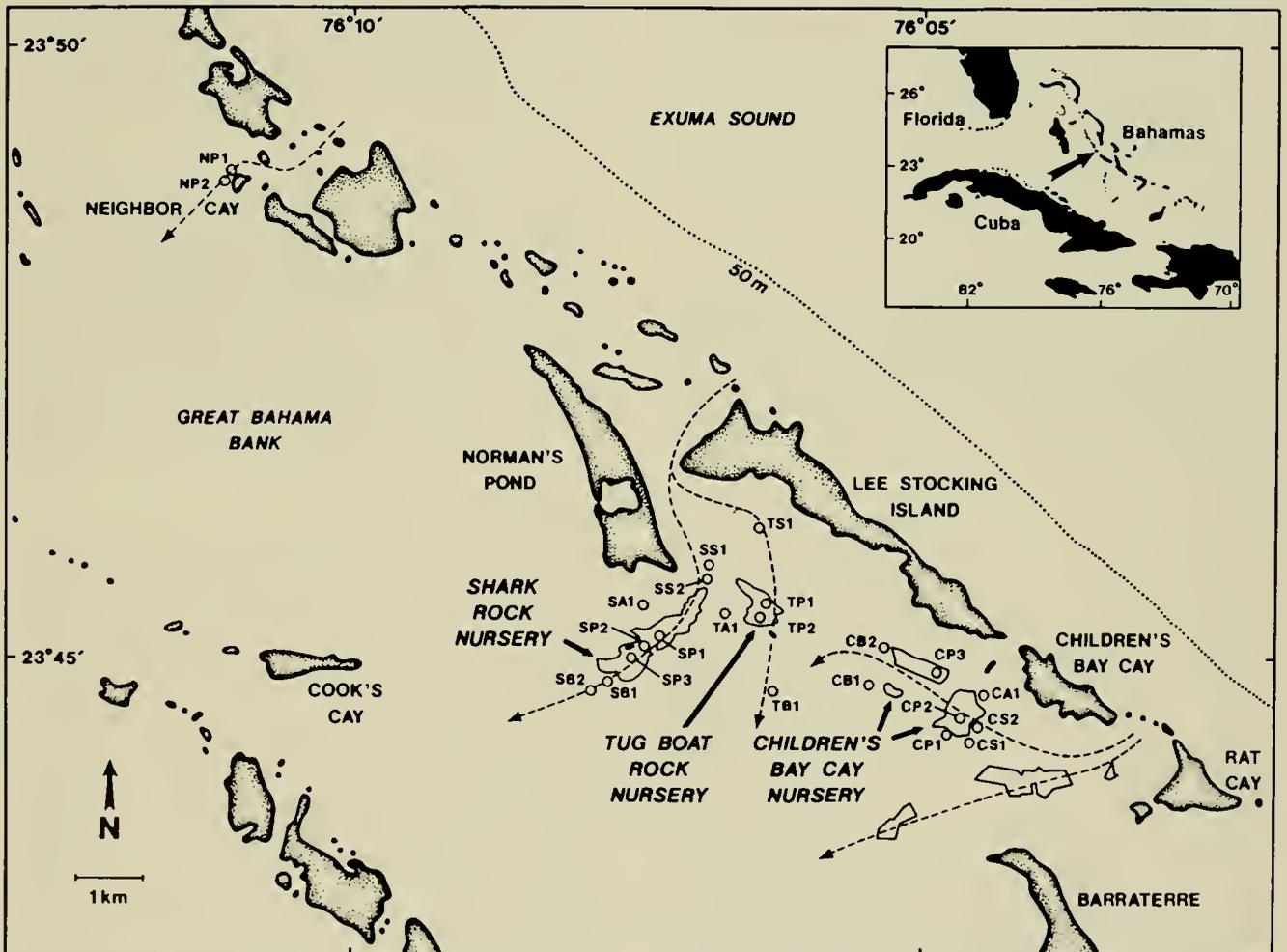


Figure 1. Map of the study sites near Lee Stocking Island in the southern Exuma Cays, Bahamas. Sampling stations were associated with the pathways of 4 different tidal current systems. Dashed lines indicate the general direction of flood tide currents, while solid lines delineate the historical boundaries of conch nurseries. The first letter of the station codes identifies the tidal system within which the station was placed. The second letter indicates station location relative to the nursery and tidal flow: within the population location (P), in the direction of the Exuma Sound (S), in the direction of the bank (B), or across the tidal flow field (A). The juvenile population at Neighbor Cay was small, and stations were located only within the population.

Cay there were 3 stations located within the nurseries, 2 stations on the sound side, 2 on the bank side, and 1 across current. At Tugboat Rock there were 2 stations within the nursery, 1 on the sound side, 1 on the bank side, and 1 across current. At the small Neighbor Cay site there were only 2 stations, both placed within the nursery. Besides the placement criteria related to current trajectory over nursery sites, stations inside and outside the nurseries were also matched as closely as possible in terms of depth, seagrass shoot density, and obvious sediment characteristics such as grain-size and general texture (e.g., firmness). Our objective was to determine what characteristics or combinations of characteristics could be used to explain the simultaneous abundance and lack of juvenile conch in seemingly similar benthic environments.

Three digit station codes, used throughout the text, tables, and figures, identify the general site, placement relative to the nursery and tidal flow, and replicate number. The first letter of the code is for site, where S = Shark Rock, T = Tugboat Rock, C = Children's Bay Cay, and N = Neighbor Cay. The second letter indicates whether the station was located within a population (P), in the direction of the sound (S), in the direction of the bank (B), or across the flow field (A). The third digit is a number which identifies replicates. For example, the code SP2 refers to station 2 within the juvenile population at Shark Rock.

#### Dependent Variables

Two measures of conch abundance were used in this study. Juvenile conch density was estimated by counting the number of individuals in 3 replicate circles (diameter = 8 m) placed haphazardly at each of the stations standardizing to number of conch 10 m<sup>-2</sup>. Aggregations of conch move about continuously, however, and any one survey represents a temporary condition in the distributional pattern. Knowledge of long-term population distribution and abundance would be more valuable than these short-term surveys in evaluation of environmental characteristics critical for supporting a conch nursery. Stoner and Ray (in review) have shown recently that the number of conch shell fragments on the surface of the sediment was directly correlated with conch density over a 3-year period at stations near Lee Stocking Island. The pattern held across 2 different tidal systems, in the Shark Rock system and in the Children's Bay Cay area. As a result of this observation, shell fragment data were used in this study to provide a long-term integration of conch density for the stations.

Density of conch shell fragments was estimated in each of the replicated circular plots described above. In each circle the sediment surface was carefully searched for pieces of shell material larger than approximately 1 cm diameter. *S. gigas* is the most abundant large gastropod on Great Bahama Bank near Lee Stocking Island and shell fragments from this species were readily identified by pink coloration on the inner surface. Complete empty shells and shell pieces were each given a count of one. This variable formed the primary basis for analyses and interpretation of relationships between long-term conch distribution and habitat characteristics.

#### Independent Variables

There are innumerable variables (both biotic and abiotic) that could be measured in an attempt to predict quality for juvenile conch. Based upon prior knowledge of variables that may affect distribution in queen conch (Randall 1964, Weil and Laughlin 1984, Stoner and Waite 1990, Stoner et al. 1994, Sandt and Stoner 1993, and others), emphasis was placed on characteristics of bank

geomorphology, sediment quality, macrophyte cover, and algal production.

Distances (to the nearest 0.1 km) between stations and the Exuma Sound were measured on topographic maps published by the Bahamas Lands and Surveys Department (1:25,000 scale) following known tidal trajectories (Stoner et al. 1995). Water depth (to the nearest 0.1 m) at each station was measured with a weighted tape and corrected for mean low water (MLW).

Two replicate cores of sediment (40 mm diameter, 5 cm depth) were collected at each station in May 1990. One subsample of ~100 g wet weight was dried at 80°C to constant weight and incinerated at 550°C for 4 hours to determine sediment organic content (percent difference between dry weight and ash-free dry weight). Another subsample of ~50 g was used to determine grain size frequency distributions. After washing to remove salts and to extract the silt-clay fraction, sand-sized particles were analyzed using standard dry sieve fractionation procedures (Folk 1966). Because the silt-clay fraction comprised <8% of the total sediment dry weight in all cases, the fraction was considered as a single unit. Product moment statistics were used to calculate mean grain size and sorting coefficients (McBride 1971).

Four replicate samples of seagrasses and macroscopic detritus (mostly senescent seagrass blades and debris) were collected from 25 × 25 cm quadrats placed haphazardly at each station in May 1990. The number of seagrass shoots within the quadrat were counted and then collected along with detritus into nylon bags with 3 mm mesh. In the laboratory detritus and above-ground parts of seagrasses (primarily *Thalassia testudinum*) were separated and dried at 80°C for ~24 hours to constant weight. Dry-weight biomass was determined for the individual components. Another 5 replicate quadrats (25 × 25 cm) were sampled similarly for macroalgae, and algal biomass was determined using the same methods as for the seagrasses. Number of macroalgal species were counted. The seagrass epiphyte loads in the study area are very light, typically less than 6% of seagrass dry weight (Stoner, Ray, and Waite 1995) and therefore the epiphytes were not separated from either seagrass or macroalgae samples.

Because macroalgae are important foods of queen conch (Robertson 1961, Randall 1964, Stoner and Waite 1991) and algal standing crop is influenced by the presence of queen conch (M. D. Hanisak, unpubl. data), algal biomass in the absence of conch was also estimated. To accomplish this, coral rock plates (15 × 15 cm square, 2.5 cm thick) were drilled through the center and suspended horizontally 30 cm above the bottom on steel reinforcement bar (diameter = 1 cm) driven into the bottom. The plates were secured in place with nylon cable ties. Four replicate plates were installed at each station in May 1990, and the accumulations of algae were harvested from the tops and sides of the plates in August 1990. Macroalgae were separated to species and dried to constant weight at 80°C. Although values for algal growth on the plates have little direct meaning in and of themselves, they provide a relative index of net population production of algae at the study sites.

Conch juveniles are frequently found in areas with strong reversing tidal currents. Since water temperatures were very uniform among the study stations, dissolution of a plaster-of-paris (calcium sulfate) block would provide a relative index of total water movement at a particular site. For this study, cylindrical blocks (~100 g) were made with wooden applicator sticks through the centers. Two blocks, dried overnight at 80°C and pre-weighed, were secured vertically to reinforcement bars at each station in September 1990. The blocks were recovered from the water, dried, and re-

weighed after 48 hours, during which time losses were between 40 and 70%. Block dissolution was quantified as grams lost per hour.

### Statistical Methods

Ordination of sampling sites on the basis of habitat descriptors was achieved using principal component analysis (PCA). The correlation matrix was used because the independent variables were measured in different units. Analysis were conducted with untransformed data as transformations did not improve the distribution of residuals and correlation coefficients.

## RESULTS

### Station Characteristics: Conch Abundance and Environmental Variables

Data on the abundance of live conch and shell fragments show that, in general, stations inside the nurseries had higher densities of live conch and/or shell fragments than the surrounding stations (Table 1). Density of shell fragments was particularly high at Children's Bay Cay sites, reflecting the high density of live conch in the area ( $>1$  conch  $m^{-2}$  at stations CP1 and CB2). When all sites were pooled, however, density of shell fragments and live conch were only weakly correlated ( $r^2 = 0.255$ ,  $p = 0.014$ ).

Data on abiotic habitat variables are summarized in Table 2. At Shark Rock and Tugboat Rock, sites within the populations were approximately 4 km from the Exuma Sound (mean = 4.5 and 4.2 km, respectively). The distance was less at Children's Bay Cay and Neighbor Cay (2.9 and 2.4 km, respectively). Average water

depth was similar among the sites except that Neighbor Cay stations were shallow. Current velocity, as indicated by dissolution of calcium sulfate blocks, varied relatively little over the study sites, except that it was low at Neighbor Cay stations. Sediment grain-size was largest (lowest values) at Neighbor Cay stations, which consisted of coarse sands ( $>0.5$  mm). There was little variation among the other stations, all of which had fine to medium sands (0.15–0.5 mm). Sorting coefficients were all in the moderate range (Folk 1966). Sediment organic content was low (between 2.18 and 4.18% of dry sediment weight) in the study site. Average organic content was slightly higher at SR than at the other sites, but no other patterns were apparent.

Macrophyte data for the 23 stations (Table 3) also showed few clear trends distinguishing primary nursery sites from the more peripheral ones. However, highest values for algae colonizing suspended rock plates occurred at nursery sites at Shark Rock, Tugboat Rock, and Children's Bay Cay. At all 3 sites net production of algae was more than 12 g dry wt plate $^{-1}$  within the populations (except at CP1), and it generally decreased with distance from the population center. Algal net production was low at Neighbor Cay as was seagrass shoot density and biomass, and detritus. Standing crop of algae on the bottom was highly variable both among and within sites. Among the 3 nursery stations at Shark Rock mean benthic algal biomass ranged from 0.11 to 12.89 g dry wt  $m^{-2}$ .

### Principal Component Analysis

The 23 sampling sites were ordinated with PCA using 14 habitat descriptors. The factor loadings of the 14 descriptors in the first 3 principal component axes are shown in Table 4. Axes 1 (high scores represent high seagrass biomass and density, high sediment organics and sorting), 2 (high scores represent high algal biomass on plates), and 3 (high scores represent high numbers of algal species) accounted for 32.1, 22.8, and 15.8% of the variance, respectively. There was a large spread of points along both axis 1 and axis 2 (especially Shark Rock and Children's Bay Cay stations) (Fig. 2), suggesting that there was considerable variation in habitat characteristics. Shark Rock and Children's Bay Cay stations mirrored one another along axis 1 (Fig. 2). Stations within both sites were spread along axis 1, with large variation in seagrass abundance. Most of the stations at Shark Rock had positive axis 2 scores, with high plate algal biomass and high number of algal species. All stations at Children's Bay Cay had negative axis 2 scores, with low algal biomass. All stations at Neighbor Cay and Tugboat Rock (except station TS1) had negative axis 1 scores, with low seagrass abundance and low organics (Fig. 2). Station TS1 had high seagrass abundance (both biomass and shoot density) (Table 3).

A total of 10 stations had positive axis 1 scores and these include 7 out of 8 stations with highest conch fragment densities (stations SP1, SP2, SP3, CP1, CP2, CS1, and CS2) (Fig. 2). Station CP3 had the fourth highest fragment density and had a small negative axis 1 score. All of these stations were located within the Shark Rock aggregation, or within or close to the Children's Bay Cay aggregation (CS1 and CS2) (Fig. 1). Other stations assumed to be within juvenile aggregations (TP1, TP2, NP1, and NP2) had low axis 1 scores and positive axis 2 scores (Fig. 2). Except for station CP3, none of the stations with negative scores on axes 1 and 2 were within juvenile aggregations or had high shell fragment densities.

All of the sites with negative scores in axis 1 and located within population centers (stations TP1, TP2, and CP3) also had negative

TABLE 1.  
Mean population density ( $\pm$ SE) of juvenile queen conch at 23 stations near Lee Stocking Island, Exuma Cays, Bahamas.

Site/Station	Live Conch (no. 10 $m^{-2}$ )	Shell Fragments (no. 10 $m^{-2}$ )
Shark Rock		
SS1	0 $\pm$ 0	1.3 $\pm$ 0.7
SS2	0 $\pm$ 0	7.4 $\pm$ 1.1
SP1	0.47 $\pm$ 0.24	18.0 $\pm$ 5.8
SP2	0.24 $\pm$ 0.24	22.4 $\pm$ 1.9
SP3	0 $\pm$ 0	18.4 $\pm$ 1.8
SB1	0.35 $\pm$ 0.01	2.0 $\pm$ 0.5
SB2	0.12 $\pm$ 0.01	1.2 $\pm$ 0.3
SA1	0 $\pm$ 0	0.8 $\pm$ 0.2
Children's Bay Cay		
CS1	0.24 $\pm$ 0.24	46.7 $\pm$ 3.4
CS2	0 $\pm$ 0	29.9 $\pm$ 3.4
CP1	12.50 $\pm$ 0.87	67.9 $\pm$ 8.3
CP2	8.00 $\pm$ 2.10	90.40 $\pm$ 2.4
CP3	6.10 $\pm$ 2.10	45.5 $\pm$ 3.9
CB1	0.12 $\pm$ 0.12	4.8 $\pm$ 2.5
CB2	13.50 $\pm$ 5.50	10.4 $\pm$ 4.2
CA1	0 $\pm$ 0	7.9 $\pm$ 0.9
Tugboat Rock		
TS1	0 $\pm$ 0	0.5 $\pm$ 0.1
TP1	2.71 $\pm$ 0.85	17.7 $\pm$ 1.8
TP2	0 $\pm$ 0	3.6 $\pm$ 0.8
TB1	0.35 $\pm$ 0.20	8.4 $\pm$ 1.6
TA1	0.24 $\pm$ 0.24	6.6 $\pm$ 1.0
Neighbor Cay		
NP1	0.20 $\pm$ 0.19	12.3 $\pm$ 2.1
NP2	10.00 $\pm$ 1.16	8.8 $\pm$ 1.7

TABLE 2.

Summary of geographic, physical, and sediment characteristics at 23 stations near Lee Stocking Island, Exuma Cays, Bahamas. Values are mean  $\pm$  SE.

Site/Station	Distance from Inlet (km)	Depth (MLW) (m)	CaSO <sub>4</sub> Dissolution (g h <sup>-1</sup> )	Sediment Grain-Size	Sediment Sorting	Sediment Organics (% dry wt)
Shark Rock						
SS1	3.4	2.0	1.31 $\pm$ 0.10	2.23 $\pm$ 0.10	1.11 $\pm$ 0.06	3.32 $\pm$ 0.28
SS2	3.6	3.0	1.26 $\pm$ 0.09	2.09 $\pm$ 0.12	1.29 $\pm$ 0.07	2.92 $\pm$ 0.08
SP1	4.3	3.1	1.23 $\pm$ 0.02	2.18 $\pm$ 0.11	1.38 $\pm$ 0.17	3.35 $\pm$ 0.13
SP2	4.5	2.9	1.12 $\pm$ 0.01	2.02 $\pm$ 0.29	1.18 $\pm$ 0.02	3.20 $\pm$ 0.01
SP3	4.7	2.9	1.21 $\pm$ 0.08	2.32 $\pm$ 0.25	1.27 $\pm$ 0.10	3.18 $\pm$ 0.24
SB1	5.0	1.6	1.06 $\pm$ 0.01	1.80 $\pm$ 0.02	1.15 $\pm$ 0.15	2.56 $\pm$ 0.08
SB2	5.4	1.7	1.09 $\pm$ 0.11	1.65 $\pm$ 0.02	1.12 $\pm$ 0.01	2.27 $\pm$ 0.06
SA1	4.0	1.6	1.17 $\pm$ 0.03	1.57 $\pm$ 0.13	1.25 $\pm$ 0.05	3.02 $\pm$ 0.08
Children's Bay Cay						
CS1	2.1	2.5	1.42 $\pm$ 0.06	1.88 $\pm$ 0.10	1.39 $\pm$ 0.03	2.93 $\pm$ 0.52
CS2	2.1	3.5	1.34 $\pm$ 0.08	1.15 $\pm$ 0.11	1.62 $\pm$ 0.19	2.80 $\pm$ 0.24
CP1	2.5	2.5	1.38 $\pm$ 0.13	1.54 $\pm$ 0.05	1.21 $\pm$ 0.15	2.61 $\pm$ 0.07
CP2	2.5	3.4	1.30 $\pm$ 0.04	2.69 $\pm$ 0.45	1.76 $\pm$ 0.09	4.18 $\pm$ 0.20
CP3	2.9	2.9	1.27 $\pm$ 0.03	1.73 $\pm$ 0.16	1.10 $\pm$ 0.06	2.57 $\pm$ 0.07
CB1	3.3	3.3	1.30 $\pm$ 0.04	1.86 $\pm$ 0.09	1.02 $\pm$ 0.02	2.51 $\pm$ 0.11
CB2	3.2	3.1	1.35 $\pm$ 0.00	1.57 $\pm$ 0.05	1.13 $\pm$ 0.01	2.44 $\pm$ 0.11
CA1	2.4	2.2	1.33 $\pm$ 0.04	1.19 $\pm$ 0.02	1.04 $\pm$ 0.01	2.66 $\pm$ 0.02
Tugboat Rock						
TS1	3.0	2.4	1.23 $\pm$ 0.03	2.50 $\pm$ 0.09	1.04 $\pm$ 0.07	2.93 $\pm$ 0.22
TP1	4.1	2.0	1.06 $\pm$ 0.06	2.04 $\pm$ 0.01	0.93 $\pm$ 0.03	2.43 $\pm$ 0.01
TP2	4.3	2.4	1.22 $\pm$ 0.04	1.88 $\pm$ 0.10	1.05 $\pm$ 0.05	2.50 $\pm$ 0.18
TB1	5.5	2.9	1.30 $\pm$ 0.02	1.89 $\pm$ 0.06	0.89 $\pm$ 0.01	2.18 $\pm$ 0.01
TA1	4.0	2.3	1.18 $\pm$ 0.20	1.80 $\pm$ 0.36	0.90 $\pm$ 0.03	2.44 $\pm$ 0.22
Neighbor Cay						
NP1	2.4	1.6	0.96 $\pm$ 0.11	1.29 $\pm$ 0.08	1.12 $\pm$ 0.09	2.65 $\pm$ 0.14
NP2	2.5	1.6	0.94 $\pm$ 0.02	1.44 $\pm$ 0.24	1.13 $\pm$ 0.05	2.73 $\pm$ 0.16

axis 3 scores (Fig. 3). Axis 3 clearly separated population stations at Tugboat Rock (TP1 and TP2) from those at Neighbor Cay (NP1 and NP2) where shell fragment densities were low, and populations of juvenile conch were ephemeral.

Summarizing the patterns observed: (1) All but one of the stations with highest shell fragment densities had strongly positive axis 1 scores (high seagrass abundance and sediment organics). (2) Areas with negative axis 1 scores but with historic records of high conch density had positive axis 2 scores and/or negative axis 3 scores (high algal biomass on plates, and low numbers of algal species in the sediment). (3) Stations with historic records of low conch density had low axis 1 scores, coupled with either low axis 2 or high axis 3 scores. These suboptimal conditions characterized all of the stations located across the flow field from traditional nursery grounds (CA1, SA1, TA1) and all of those on the bank side of the nurseries (CB1, CB2, SB1, SB2, and TB1). On the other hand, three of the stations surveyed (SS1, SS2, and TS1) had seemingly optimal environmental conditions (Figs. 2 and 3), but low shell fragment densities, indicating relatively low occupancy over the long term (Table 1). All of these stations were located between the traditional nursery grounds and Exuma Sound (Fig. 1) (Table 2).

## DISCUSSION

Principal component analysis indicated that no single habitat variable was a good predictor of juvenile conch abundance at the 23 stations surveyed. As has been shown in numerous investiga-

tions, seagrass biomass was an important component of the distributional relationship, but conch were not always associated with high seagrass biomass, and sediment organics, detritus, algal growth, and number of algal species in the sediment were all important elements in conch distribution. Other multivariate analyses of large-scale faunal distributions in seagrass habitats show some similarities to our analyses. For example, Sogard et al. (1987) found that seagrass abundance was an important variable associated with the density of epibenthic fishes in Florida Bay seagrass meadows, but seagrass detritus, water depth, and sediment characteristics also contributed significantly to the variation in abundance. In this study, algal net production in the fouling plates was more closely associated with conch distribution than algal standing crop. The biomass of macroalgae is strongly influenced by conch grazing (Stoner 1989, Stoner and Hanisak, unpubl. data), perhaps explaining the lack of direct relationship to conch distribution.

The exact mechanisms of the association between juvenile conch distribution and environmental variables identified in this investigation remain unknown; however, the large-scale associations could be related to differences in (1) larval supply, (2) settlement behavior, (3) habitat preferences in juvenile conch, and/or (4) differential survivorship. Stoner and Davis (in review) have shown that conch veligers are more concentrated over the traditional nursery ground at Shark Rock than at stations located either further onto the bank or closer to Exuma Sound. Tidal transport of veligers from offshore spawning grounds to the nurseries almost

TABLE 3.

Summary of benthic macrophyte characteristics at 23 stations near Lee Stocking Island, Exuma Cays, Bahamas. Values are mean  $\pm$  SE.

Site/Station	<i>Thalassia</i> Shoots (no. m <sup>-2</sup> )	<i>Thalassia</i> Biomass (g dry m <sup>-2</sup> )	Detritus (g dry wt m <sup>-2</sup> )	Total Algal Biomass (g dry wt m <sup>-2</sup> )	<i>Batophora</i> Biomass (g dry wt m <sup>-2</sup> )	Algal Species (no. sample <sup>-1</sup> )	Algal Biomass on Plates (g dry wt)	<i>Batophora</i> Biomass on Plates (g dry wt)
Shark Rock								
SS1	40 $\pm$ 1	3.76 $\pm$ 0.35	4.96 $\pm$ 0.95	6.83 $\pm$ 1.58	5.12 $\pm$ 1.50	5 $\pm$ 1	7.59 $\pm$ 1.02	7.21 $\pm$ 1.04
SS2	38 $\pm$ 3	4.36 $\pm$ 0.36	7.02 $\pm$ 1.99	1.19 $\pm$ 0.47	0.66 $\pm$ 0.24	2 $\pm$ 1	13.66 $\pm$ 2.50	13.64 $\pm$ 2.50
SP1	40 $\pm$ 3	3.88 $\pm$ 0.30	3.16 $\pm$ 1.11	12.89 $\pm$ 1.16	10.82 $\pm$ 0.85	6 $\pm$ 1	16.38 $\pm$ 4.15	16.31 $\pm$ 4.14
SP2	31 $\pm$ 3	2.45 $\pm$ 0.26	7.56 $\pm$ 4.21	4.54 $\pm$ 0.88	3.96 $\pm$ 1.08	2 $\pm$ 0	34.94 $\pm$ 2.19	34.94 $\pm$ 2.19
SP3	36 $\pm$ 2	4.23 $\pm$ 0.35	8.22 $\pm$ 3.50	0.11 $\pm$ 0.07	0.07 $\pm$ 0.05	2 $\pm$ 0	31.18 $\pm$ 2.14	31.18 $\pm$ 2.14
SB1	31 $\pm$ 3	1.92 $\pm$ 0.21	0.74 $\pm$ 0.24	1.82 $\pm$ 0.71	1.15 $\pm$ 0.56	3 $\pm$ 1	15.61 $\pm$ 6.25	15.35 $\pm$ 6.27
SB2	17 $\pm$ 3	1.40 $\pm$ 0.23	0.53 $\pm$ 0.16	4.85 $\pm$ 1.62	0.97 $\pm$ 0.25	5 $\pm$ 1	15.82 $\pm$ 4.47	15.82 $\pm$ 4.47
SA1	49 $\pm$ 2	1.75 $\pm$ 0.15	1.15 $\pm$ 0.43	0.15 $\pm$ 0.04	0.03 $\pm$ 0.01	2 $\pm$ 0	0.04 $\pm$ 0.04	0.04 $\pm$ 0.04
Children's Bay Cay								
CS1	50 $\pm$ 6	4.01 $\pm$ 0.16	6.61 $\pm$ 0.64	6.23 $\pm$ 0.88	6.04 $\pm$ 0.77	3 $\pm$ 0	12.36 $\pm$ 0.50	12.22 $\pm$ 0.05
CS2	54 $\pm$ 4	6.11 $\pm$ 0.71	10.65 $\pm$ 2.07	1.06 $\pm$ 0.52	0.73 $\pm$ 0.30	3 $\pm$ 1	3.71 $\pm$ 1.55	3.64 $\pm$ 1.59
CP1	46 $\pm$ 4	4.17 $\pm$ 0.81	16.54 $\pm$ 3.45	1.24 $\pm$ 0.52	0.97 $\pm$ 0.40	2 $\pm$ 1	4.59 $\pm$ 2.48	4.51 $\pm$ 2.50
CP2	59 $\pm$ 8	3.68 $\pm$ 0.71	6.78 $\pm$ 0.86	0.61 $\pm$ 0.38	0.05 $\pm$ 0.04	1 $\pm$ 1	17.82 $\pm$ 6.00	17.78 $\pm$ 6.02
CP3	37 $\pm$ 5	2.62 $\pm$ 0.18	1.36 $\pm$ 0.60	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	14.80 $\pm$ 5.86	14.41 $\pm$ 5.97
CB1	45 $\pm$ 5	2.84 $\pm$ 0.52	2.61 $\pm$ 1.01	0.43 $\pm$ 0.12	0.38 $\pm$ 0.12	2 $\pm$ 1	0.15 $\pm$ 0.11	0.11 $\pm$ 0.11
CB2	36 $\pm$ 7	1.63 $\pm$ 0.22	0.51 $\pm$ 0.17	0.13 $\pm$ 0.13	0.08 $\pm$ 0.08	1 $\pm$ 0	5.46 $\pm$ 2.80	4.85 $\pm$ 3.20
CA1	31 $\pm$ 2	1.42 $\pm$ 0.15	2.76 $\pm$ 1.41	0.43 $\pm$ 0.09	0.34 $\pm$ 0.09	3 $\pm$ 1	2.23 $\pm$ 1.01	1.83 $\pm$ 1.12
Tugboat Rock								
TS1	42 $\pm$ 4	3.37 $\pm$ 0.41	6.85 $\pm$ 2.52	0.07 $\pm$ 0.04	0 $\pm$ 0	1 $\pm$ 1	4.06 $\pm$ 1.54	3.86 $\pm$ 1.58
TP1	22 $\pm$ 8	1.35 $\pm$ 0.43	2.13 $\pm$ 0.96	0.10 $\pm$ 0.50	0.03 $\pm$ 0.01	2 $\pm$ 1	16.24 $\pm$ 2.50	16.24 $\pm$ 2.50
TP2	13 $\pm$ 2	1.93 $\pm$ 0.25	2.12 $\pm$ 0.73	0.76 $\pm$ 0.29	0.23 $\pm$ 0.06	2 $\pm$ 0	12.14 $\pm$ 3.68	12.08 $\pm$ 3.67
TB1	23 $\pm$ 2	2.00 $\pm$ 0.39	0.38 $\pm$ 0.16	0.05 $\pm$ 0.04	0.05 $\pm$ 0.04	1 $\pm$ 1	8.61 $\pm$ 3.23	8.61 $\pm$ 3.23
TA1	37 $\pm$ 7	2.24 $\pm$ 0.54	0.37 $\pm$ 0.10	0.15 $\pm$ 0.08	0.13 $\pm$ 0.06	1 $\pm$ 1	1.04 $\pm$ 0.34	0.89 $\pm$ 0.33
Neighbor Cay								
NP1	19 $\pm$ 5	1.02 $\pm$ 0.40	0.71 $\pm$ 0.25	0.54 $\pm$ 0.03	0.06 $\pm$ 0.03	4 $\pm$ 1	5.47 $\pm$ 1.71	5.20 $\pm$ 1.75
NP2	25 $\pm$ 3	1.38 $\pm$ 0.26	1.71 $\pm$ 0.37	0.61 $\pm$ 0.39	0.08 $\pm$ 0.07	4 $\pm$ 1	4.22 $\pm$ 1.70	4.04 $\pm$ 1.63

certainly explains the association of nurseries with tidal current systems (Stoner et al. 1995) and may concentrate the larvae at traditional nursery sites.

Competent queen conch larvae settle and metamorphose in response to very specific compounds found in macroalgae such as *Laurencia* spp. and *Batophora oerstedii* (Mianmanus 1988, Davis and Stoner 1994). These were the dominant algae in the seagrass

TABLE 4.

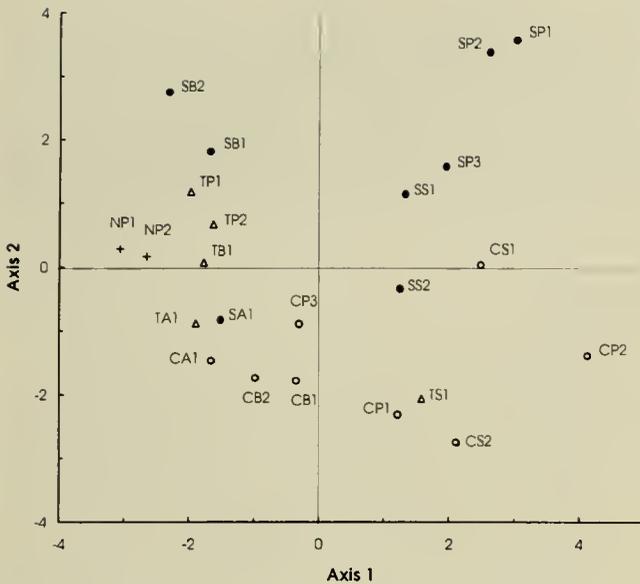
The factor loadings of the habitat descriptors in the first 3 principal component axes.

Descriptor	Axis 1	Axis 2	Axis 3
Distance from Inlet (km)	-0.297	-0.661	0.329
Depth (MLW, m)	0.670	0.226	0.343
CaSO <sub>4</sub> Dissolution (gh <sup>-1</sup> )	0.582	0.474	0.015
Sediment Grain-Size	0.481	-0.384	0.433
Sediment Sorting	0.796	0.069	-0.108
Sediment Organics (% dry wt)	0.773	-0.177	0.008
<i>Thalassia</i> Shoots (no. m <sup>-2</sup> )	0.764	0.470	-0.074
<i>Thalassia</i> Biomass (g dry wt m <sup>-2</sup> )	0.865	0.208	-0.031
Detritus (g dry wt m <sup>-2</sup> )	0.704	0.242	0.068
Total Algal Biomass (g dry wt m <sup>-2</sup> )	0.436	-0.613	-0.591
<i>Batophora</i> Biomass (g dry wt m <sup>-2</sup> )	0.507	-0.528	-0.557
Algal Species (no. sample <sup>-1</sup> )	0.041	-0.498	-0.795
Algal Biomass on Plates (g dry wt)	0.346	-0.736	0.488
<i>Batophora</i> Biomass on Plates (g dry wt)	0.348	-0.736	0.490

areas known to be historically important for juvenile conch, but were distributed independently from the seagrasses. Net production of algae on the fouling plates (primarily *B. oerstedii*) also reached maxima within the traditional nursery areas (Stoner et al. 1994, this study). Substrata collected from within the Shark Rock nursery area elicited much higher metamorphic responses than substrata collected from surrounding areas, even when these substrata, such as seagrass detritus and fronds of *B. oerstedii* appeared to be similar (Davis and Stoner 1994). It is very likely, therefore, that the observed distribution patterns are affected by differential settlement of larvae, which is related to presence of particular substrata or microflora associated with the substrata.

Choice of habitat within seagrass meadows has been tested experimentally with small invertebrates (Stoner 1980b), fishes (Steffe et al. 1989, Bell and Westoby 1986), and juvenile queen conch (Stoner and Waite 1990). Given that predation rates on queen conch juveniles decrease with increasing seagrass structure (Marshall 1992, Ray and Stoner 1994), it is likely that the preference of juvenile conch for particular seagrass densities (Stoner and Waite 1990) is an evolved behavioral adaptation. Similarly, queen conch may have other habitat preferences that affect distributional patterns, such as preferences for detritus, specific algae, or organic compounds in the sediment.

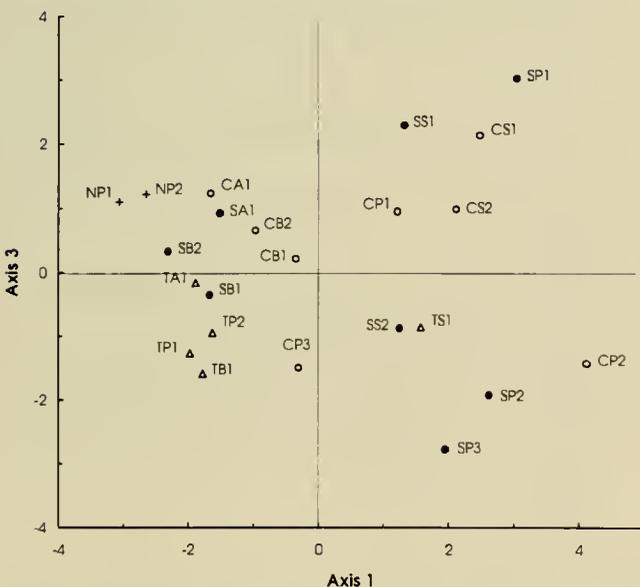
The observed distributional pattern may also be related to differential survivorship of post-settlement conch. Field experiments have shown that survival rate in small conch is influenced by habitat structure (Marshall 1992, Ray and Stoner 1994), but may also be site specific (Stoner and Sandt 1991, Stoner et al. 1994).



**Figure 2.** Principal component analysis of environmental characteristics at the sampling stations on Great Bahama Bank. High scores on Axis 1 indicate high seagrass biomass, sediment organics and sorting. High scores on Axis 2 indicate high algal biomass on fouling plates. Station codes (described in Figure 1) are shown beside each point.

Also, mortality rates in juvenile queen conch are inversely density-dependent (Marshall 1992, Ray and Stoner 1994, 1995).

On the basis of principal component analysis, there were a few stations that appeared to have suitable conditions for juvenile queen conch but showed little evidence of long-term occupation by the species. Invariably, these stations were located in the direction of the Exuma Sound from the historically important nursery sites. Poor larval supply or retention could explain the lack of conch in these seaward seagrass beds because veliger concentrations are significantly higher at locations further onto the bank (Stoner,



**Figure 3.** Principal component analysis of environmental characteristics at the sampling stations on Great Bahama Bank. High scores on Axis 1 indicate high seagrass biomass, sediment organics and sorting. High scores on Axis 3 indicate high numbers of algal species. Station codes (described in Figure 1) are shown beside each point.

unpubl. data). Experimental evidence suggests, however, that the habitat is not appropriate for juvenile conch. Transplants of juvenile conch to two of the sites examined in this investigation (TS1 and SS2) (Stoner and Sandt 1991, and Stoner et al. 1994, respectively) resulted in significantly lower survivorship and growth rates than those measured simultaneously in nearby nurseries. This suggests that there were station differences in both predator-prey relationships and nutritional quality. Predator suites undoubtedly vary spatially, and some unmeasured variable related to tidal flushing or nutrient cycling may explain the poor nutritional quality of these seemingly appropriate habitats. Substrata from these same locations also failed to elicit metamorphosis in competent larvae (Davis and Stoner 1994).

Stations considered to be located within queen conch populations at Tugboat Rock and Neighbor Cay were distant, along the principal component axes, from the larger, more densely populated Shark Rock and Children's Bay Cay nursery locations. A long-term record of population size at Tugboat Rock and Neighbor Cay shows that both are subject to large fluctuations in size and density (Stoner et al. 1995, unpubl. data). For example, the Tugboat Rock population was large in 1989, 1990, and 1993, but disappeared almost entirely in 1991 and 1992. At Neighbor Cay, very few conch have been seen since 1990. Both seagrass biomass and detrital loads were suboptimal at these locations, which offer suitable habitat only when algal productivity is high and when large numbers of larvae are available. We also know that algal productivity on the bank is highly variable from year to year (Hanisak, unpubl. data). Clearly, nursery grounds do not have equal quality or stability in terms of supporting juvenile queen conch.

It is also likely that different nursery areas support conch through different mechanisms. For example, one system may provide abundant food and shelter in the form of seagrass biomass and detritus, while another has high algal productivity. The significance of algae in the distribution of juvenile queen conch is illustrated by the fact that some conch nurseries occur entirely outside seagrass beds. For example, greatest concentrations of juveniles in the Florida Keys, where populations are much smaller than those in the Bahamas, are associated with hard-bottom areas with red and green macroalgae, rather than seagrasses (Berg et al. 1992, Glazer and Berg 1994). Nevertheless, this study, along with a rapidly growing literature on the ecology of queen conch, indicates that the most important queen conch nurseries are locations that have a combination of high food production (primarily macroalgae and seagrass detritus), high quality oceanic seawater, abundant larval supply, and low predation rates.

It is now clear that the general locations of queen conch nurseries are ecologically unique, and there are vast areas of seagrass meadows completely unsuitable for juvenile conch. This is evident from the fact that few transplants outside traditional nursery grounds have resulted in high survivorship and growth, and by the observation that only about 1.5% of 8,300 ha of seagrass meadow surrounding Lee Stocking Island was occupied by queen conch over the four year period from 1989 to 1992 (Stoner et al. 1995). It would be a mistake to proceed with a queen conch stock enhancement program assuming that most seagrass meadows provide adequate nursery habitat for the released stock. However, careful measurements of key variables, such as seagrass production, sediment qualities, algal growth, larval supply, and predatory rate should permit high probability of predicting suitable locations. Small-scale transplant experiments prior to outplanting should be used to further increase the chance of success (Stoner 1994). To

effectively manage natural queen conch populations and rehabilitate depleted stocks, historically significant nursery grounds must be identified and protected because of their unique qualities.

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## REPRODUCTIVE ANATOMY AND BIOLOGY OF THE GENUS *STROMBUS* IN THE CARIBBEAN: I. MALES

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**ABSTRACT** Strombid males preferred to mate with spawning females. During copulation, the female securely held the verge of the male. An infrequent, but consistent observation, was the presence of a second male during copulation. Copulation was not the same for these secondary males: the verge was not securely attached. In all 6 species, at least one incident of interspecific but otherwise normal copulation was observed, as well as incidents of males attempting to copulate with other males already engaged in copulation with a female. Male reproductive anatomy was studied macro- and microscopically for all Caribbean *Strombus* species; results were consistent among species and assumed to be applicable to the genus as a whole. Male prostatic tissue is composed of secretory cells that appear to be modified cuboidal epithelium; the sperm groove consists of ciliated epithelium and goblet cells. Verge morphology varied among species, but did not interfere with interspecific copulation.

**KEY WORDS:** Gastropoda, Strombidae, reproduction, anatomy, biology

### INTRODUCTION

The Strombidae are tropical gastropods, with the majority of species found in the Indo-Pacific region; only 7 species are present in the western Atlantic, one of which is endemic to the coast of Brazil (*Strombus goliath* Schröter 1805). Of these species, research has focused on *Strombus gigas* Linnaeus 1758 due to its commercial importance. The general anatomy of this sexually dimorphic species was studied by Little (1965), with the notable exclusion of the reproductive systems. The other 5 species present in the Caribbean are *S. costatus* Gmelin 1791, *S. raninus* Gmelin 1791, *S. gallus* Linnaeus 1758, *S. pugilis* Linnaeus 1758, and *S. alatus* Gmelin 1791.

Individual mating behaviors have been recorded but not described in detail or quantified. In *Strombus*, more than 1 male may attempt to copulate with the same female at the same time; copulation can occur concurrently with egg extrusion by the female, as well as at other times (Hesse 1979, Catterall and Poiner 1982, Kuwamura et al. 1982, pers. obs.). Also, mating preferences, such as the preference of a male for copulating with a spawning versus a non-spawning female, have not been quantified in strombids, with the exception of *S. costatus* (Appeldoorn, in press) where males were found copulating more often with spawning females. During courses of study on reproductive seasonality of *S. pugilis* (Reed 1994) and effective reproductive density in *S. gigas* (Appeldoorn, in prep.), these same behaviors were observed, but were not explicable based on the current knowledge of strombid anatomy and biology. Elaboration of male and female anatomy was needed to explain the occurrence of "primary" and "secondary" copulating males and the differences in their reproductive behavior, and to investigate the phenomenon of "guarding" as postulated by Bradshaw-Hawkins (1982).

Male strombids have a penis (termed "verge") located on the foot posterior to, and to the right of, the right cephalic tentacle. The distal end of the verge varies in shape among species and has been used as a taxonomic characteristic (Abbott 1960). The vari-

ation in verge shape among species was hypothesized to be a mechanism for the prevention of interspecific copulation and hence, crossbreeding (Abbott 1960).

Like many prosobranch gastropods, the Strombidae are characterized by the production of dimorphic spermatozoa. The typical (eupyrene) sperm are thread-like and contain chromatin in the head whereas the atypical (apyrene) sperm are vermiform and do not contain chromatin (Reinke 1912). Both types are motile. Ultrastructural study of apyrene sperm shows that they contain granules of glycoprotein and polysaccharides (Koike and Nishikawa 1980). Spermiogenesis has been studied in *S. gigas* (Egan 1985) and is comparable to other gastropod species (e.g., West 1978).

The purpose of this investigation was to complete the description of the male reproductive system using the 6 Caribbean species of the genus *Strombus*. Also, spermatozoa and verges were compared among these species, and male reproductive behavior was observed.

### METHODS

Males of *S. gigas* ( $n > 100$ ), *S. costatus* ( $n > 100$ ), *S. raninus* ( $n = 3$ ), *S. gallus* ( $n = 3$ ), and *S. pugilis* ( $n > 100$ ) were collected off the southwest coast of Puerto Rico. Live male *S. alatus* ( $n = 12$ ) were obtained from southern Florida. Animals were removed from their shells and immediately dissected in order to study internal anatomy. Also, 40 immature *S. gigas* were collected in order to look at reproductive gland development both before and after external genitalia began forming as a method for distinguishing between males and females before external genitalia develop. Of these immature conch, 19 were male.

For histological analyses, portions of the reproductive glands and verges (including 1 regenerating verge) were excised from freshly opened, unrelaxed animals (2 mature males of each species), and fixed for 24 hours in Bouin's solution. Following fixation, tissues were dehydrated in 95% ethanol and embedded in paraffin. Sections were cut 6-10  $\mu\text{m}$  in thickness and mounted on albuminized slides. Staining was with hematoxylin and eosin according to Harris' regressive method in Howard and Smith (1983).

Fresh samples of semen were taken directly from the vas deferens for study using light microscopy. Estimates of eupyrene and

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apyrene sperm size were taken as maximum length recorded, as their highly motile nature made precise measurements difficult.

Sperm samples were taken from female *S. costatus* and *S. pugilis* collected while engaged in copulation. Copulation is defined functionally here as the male conch having his verge extended under the shell of the female (the actual act of sperm transfer cannot be observed, but only inferred). A male, positioned to the rear of the female and apparently securely attached via his verge during copulation, is termed the "primary" male. Other males positioned head-on or to one side of the female, with their verges extended under her shell but not fastened in any way, are termed "secondary" males. Primary males had to be pulled apart from the female, which sometimes resulted in breakage of the verge (secondary males were readily removed, i.e., with no resistance). Samples were also taken from females dissected 1, 2, 4, and 24 hours ( $n \geq 10$  for the 2 species per time period) after copulation was completed (when the male left the female) in order to examine any changes in sperm morphology after deposition in the females. Females that had mated were kept isolated in the lab from further contact with any male for several days; samples of sperm were then removed from the receptaculum seminis of each female. Further observations of sperm contained in storage sacs were made from *S. gigas*, *S. raninus*, and *S. gallus* females collected in the field.

A mixed collection of adult *S. raninus*, *S. gallus*, and *S. costatus* males and females, as well as a separate collection of adult *S. pugilis* and *S. alatus*, were maintained in a tank with free-flowing saltwater in order to observe mating and spawning behaviors, and to obtain egg masses from the adult females. The rare species, *S. raninus* and *S. gallus*, were specifically observed for copulatory and spawning behaviors in order to verify that the behavior was the same for all species of strombids in the Caribbean. With the presence of congeners, cases of interspecific copulation not seen in the field were of specific interest, as some species are rare and seldom intermixed. Such observations are not possible in the field due to depth and bottom time restrictions when using SCUBA for research purposes. Populations of *S. pugilis* were observed in the field (Reed 1994) and reproductive behaviors were recorded on an individual basis. Particular attention was paid to the position of the copulating male(s) and to the reproductive status of the female (actively extruding egg strand or not). Other species were similarly observed when chanced upon.

## RESULTS

### General Anatomy

Male anatomy did not differ among species studied except for shape of the verge. The following description of the reproductive system thus applies to all species included in this study. Figure 1 illustrates a generalized adult male strombid with the mantle cavity opened from left to right. Various organs are labelled in order to show the relative orientation and position of reproductive structures.

The testis overlies the digestive gland at the distal end of the body, which is located in the spire of the shell when the animal is intact. Seminiferous tubules run throughout the gonad, eventually uniting to form a single duct that passes along the columellar side of the visceral mass, until it reaches the proximal edge of the prostate gland. This single vas deferens then opens into a groove formed by the prostate gland and the mantle wall. From the end of the prostate gland, this groove continues as a slight fold on the

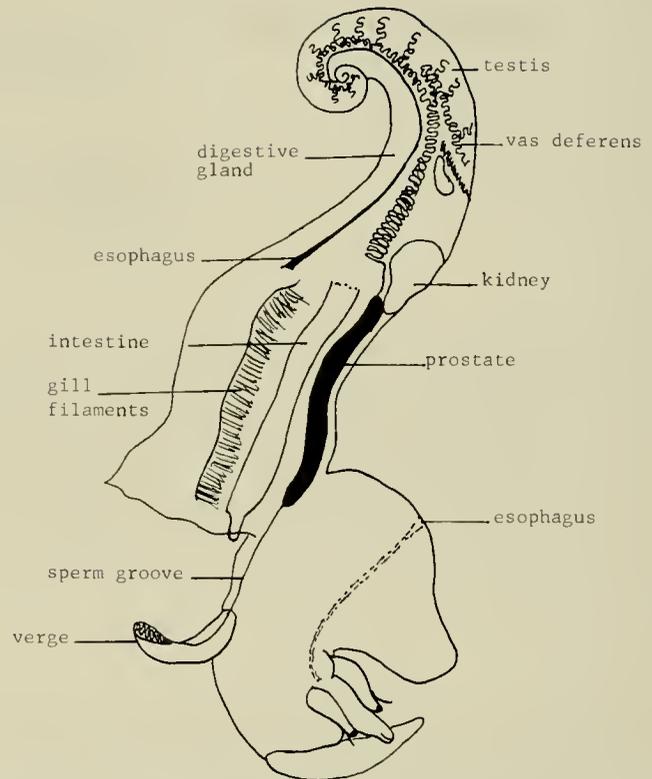


Figure 1. Schematic drawing of a generalized male *Strombus* with mantle cavity laid open to the right showing position and orientation of reproductive organs.

mantle and then extends onto the foot, where it is formed by extension of the epithelium.

The gonad and associated ducts are covered by a thin layer of pallial epithelium, as is the rest of the visceral mass, and are entirely separate from the digestive gland. The main portion of the duct leading from the testis is the functional equivalent of the vas deferens and the seminal vesicle combined, and becomes quite swollen with stored sperm when the male is reproductively active. Convolutions of the vas deferens develop when the male first begins to produce sperm. The walls appear to lack elasticity as they never shrink back to their original state even when empty as observed in reproductively inactive males collected during winter months. In immature males, all seminiferous ducts are narrow and straight.

The prostate gland lies parallel to the intestine in the visceral mass. In reproductively active males, the prostatic tissue becomes swollen, and takes on olive-green coloration. In immature males, prostatic tissue is not developed, and appears as a thin white line on the mantle wall. The prostate gland begins developing at the same time as the verge begins growing.

Testicular tissue is white in juveniles, changing to cream as maturation occurs (when the lip of the shell is formed), and finally to orange when the gonad itself has matured and sperm production is occurring. *S. gigas*, *S. pugilis*, and *S. alatus* adult males typically have bright orange gonads whereas those of *S. costatus* have yellowish-orange coloration, and of *S. gallus* and *S. raninus* have greenish-orange coloration.

Differences in the shape of the distal portion of the verges of the Caribbean species are illustrated in Figure 2, after the fashion of Abbott (1960). In *S. gigas* and *S. costatus*, the verge is a simple prong with a spade-shaped tip (Fig. 2A,B). The verge of *S. rani-*

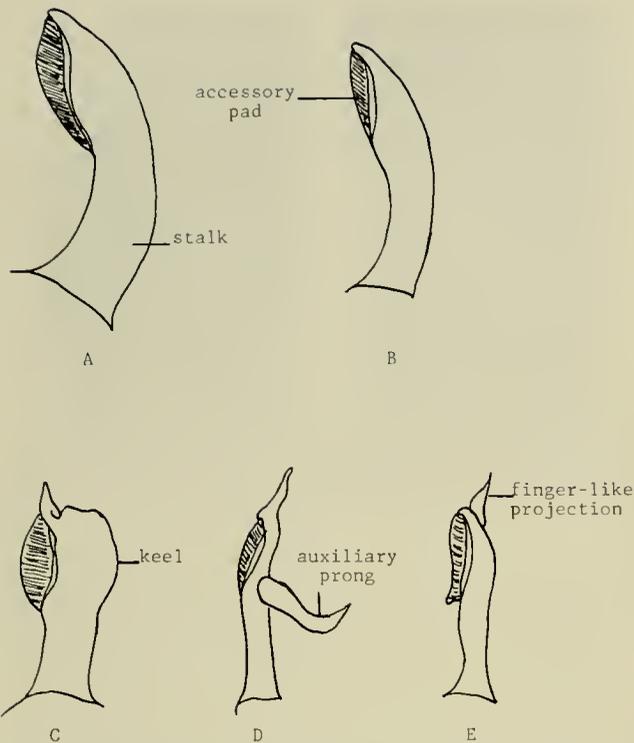


Figure 2. Verges of A) *S. gigas* ( $\times 1$ ), B) *S. costatus* ( $\times 1.5$ ), C) *S. raninus* ( $\times 2$ ), D) *S. gallus* ( $\times 2$ ), and E) *S. pugilis* and *S. alatus* ( $\times 2$ ); drawn after the fashion of Abbott (1960).

*nus* is keeled (dorsal side of the verge tip is flattened) with a club-shaped head (Fig. 2C). *S. gallus* has an auxiliary prong arising from the stalk of the verge (Fig. 2D), corresponding to the bilobed condition described by Abbott (1960). In *S. pugilis* and *S. alatus*, there is a finger-like projection from the end of the accessory pad (extended portion of verge tip that closes over the verge) (Fig. 2E); there is no morphological difference between the verges of these latter 2 species.

The sperm groove continues as a fold of the epithelium along the surface of the verge. The accessory pad of the verge opens up to reveal a hollow tip into which the groove empties when transporting sperm. Two convoluted rows of papillae are visible within the tip. The stalk of the verge is capable of stretching at least six times its length in order to reach the copulatory organ of the female.

Males that have lost their verges are able to regenerate them. The new verge grows from the stub of the old one. Figure 3 illustrates an adult male verge for comparison to a just-maturing (when the flared lip of the shell is forming; see Appeldoorn 1988 for immature male genital development) male's and to one that is regenerating. A thin band of scar tissue is noticeable in males that have lost and regrown new ones. There appears to be no impairment of function once regrowth is complete, as such males have been observed copulating. Although rare, male *S. gigas* ( $n = 4$ ), *S. costatus* ( $n = 6$ ), and *S. pugilis* ( $n = 11$ ) were collected that had lost their verges, and were regrowing or had regrown them.

Sperm samples taken from the different species of conch all had both apyrene and eupyrene types. Apyrene sperm were vermiform in shape and consisted of a membrane enclosing a spindle of granules (a "corn cob"). Eupyrene sperm consisted of a small spindle-shaped head and long whip-like tail, and measured ap-



Figure 3. Stages of verge development using *S. gigas* as the example ( $\times 1$ ): A) mature male, B) maturing male (flared lip of shell is forming), and C) regenerating verge; drawn after the fashion of Abbott (1960).

proximately  $5 \mu\text{m}$  for all species. Apyrene sperm were estimated to be  $114 \mu\text{m}$  for *S. gigas* and *S. gallus*,  $100 \mu\text{m}$  for *S. costatus*, *S. pugilis*, and *S. alatus*, and  $71 \mu\text{m}$  for *S. raninus*.

Apyrene sperm disintegrated within 2 hours of dissection from the vas deferens of the male, and within 2 hours of deposition in the female, regardless of where the sperm sample was taken within the female. Eupyrene sperm in the receptaculum seminis of the female were found to be immobilized within mucosal secretions. There were no visible changes in sperm morphology after deposition in the female. Results were the same for all species studied.

#### Histology

There were no discernible interspecific differences in the tissues examined; thus, results can be applied to any of the male strombids included in this study.

Longitudinal sections through the distal portion of the verge (Fig. 4) show details of lamellae on the accessory pad (A) and inner sperm groove (B). The entire surface of these lamellae is covered with ciliated epithelium, interspersed with goblet cells. In Figure 4B, 1 of the 2 distinct rows of papillae is shown. Note that goblet cells are not present in the section between the 2 rows of papillae (left side of figure). Muscle fibers are apparent running in both length- and cross-wise directions, and are arranged in distinct layers. Figure 5 shows a longitudinal section of a regenerating verge. Note that the tissue is infiltrated with leukocytes, indicating increased blood supply, probably due to the tissue damage sustained when the animal was injured.

The prostate gland (Fig. 6) is almost completely composed of secretory cells, which are arranged in single layers around a lumen. Lumina are more pronounced in a reproductively active male (A), and the cells are swollen with cytoplasm, as compared to an inactive male (B), in which the lumina are collapsed. The surface of the prostate gland is covered with ciliated epithelium interspersed with goblet cells under which runs a layer of overlapping collagen fibers (not shown). The lining of the groove is composed of ciliated epithelium interspersed with goblet cells under which runs a layer of longitudinally orientated muscle fibers of 5–7 cells thickness (upper right corner of Fig. 6B).

#### Behavioral Observations

Data collected on individual *S. pugilis* mating preference in the field showed that males ( $n = 402$ ) mated with spawning females 99.0% of the time; only 4 cases of a male copulating with a

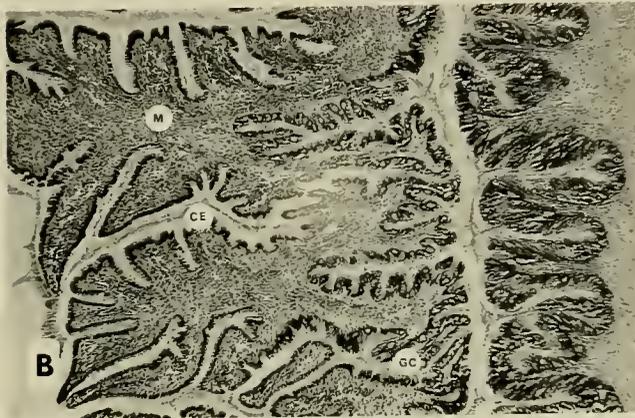
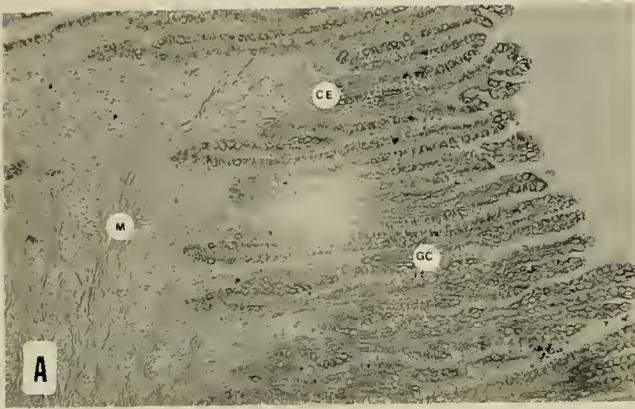


Figure 4. Histological sections of the verge: A) surface lamellae, *S. raninus*, longitudinal section ( $\times 100$ ), and B) interior lamellae, *S. gigas*, longitudinal section ( $\times 100$ ): CE, ciliated epithelium; GC, goblet cells; M, muscle.

non-spawning female were observed in the entire year and a half of field observation, and no cases were seen in the tank. Of the 398 females observed spawning, those found spawning alone accounted for 23.9%; those mating with one partner, 68.4% (or 91.1% of males); and with more than one partner, 6.7% (or 8.9% of males). Non-spawning females found copulating accounted for only 1% of the observations. There were 2 cases observed of a



Figure 5. Histological section of the regenerating verge of a male *S. costatus* ( $\times 100$ ): CE, ciliated epithelium; GC, goblet cell; M, muscle; arrows, leukocytes (black line represents break between original and regenerating verge portions).

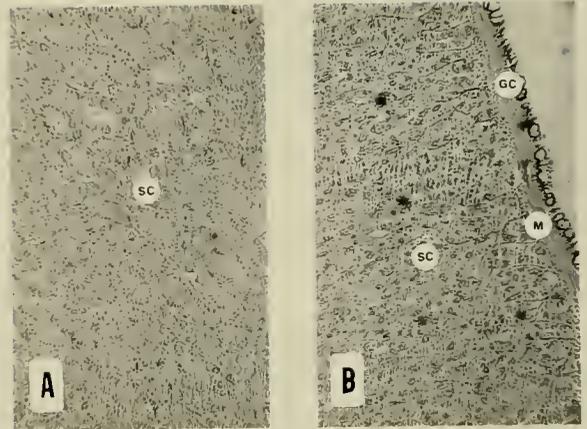


Figure 6. Longitudinal histological sections of the prostate gland: A) *S. raninus*, reproductively active male ( $\times 100$ ), B) *S. gigas*, reproductively inactive male ( $\times 100$ ): GC, goblet cell; M, muscle; SC, secretory cells.

male attempting to copulate with another male (sex verified by dissection).

In both the field and the tank, mating was promiscuous, with males found copulating with different females, sometimes within minutes of having left a female. In the tank, primary males were often observed to remain in position on their partners for the length of time the females continued spawning. Attempts to remove primary males from their partners were unsuccessful, as the males appeared to be firmly attached to the females via the verge. Pulling a primary male away from the female resulted in stretching of the verge and eventual breakage, if stretched too far. Secondary males were observed, both in the field and the tank, to change partners, apparently at random, with much variation in the time spent as the secondary male (from 1 minute to several hours). Secondary males were easily removed from their partners, with none of the resistance seen for primary males. The verge could be seen retracting under the shell of the secondary male as he was pulled away. While testing primary and secondary males by pulling, care was taken not to disturb the associated females. In no case did a female retract into her shell (which would have trapped the verge), but rather continued to spawn.

In the field, only one incident was ever observed of a male attempting to dislodge another male from the female. This male inserted the lip of his shell under the body whorl of the other and then jerked upwards. He made 3 attempts to flip the other male before moving away. At the laboratory, an episode of "sparring" was observed between two males copulating with one spawning female. They jabbed at each other with their probosci over the head of the female, who continued to spawn. Neither male retracted his verge during sparring. After 2 minutes, both males subsided and began grazing on the female's and each others' shells. This bizarre behavior apparently had more to do with feeding than with reproduction.

Although quantitative data were not recorded, the attraction of males to spawning females did not appear to be related to the size of an incomplete egg mass, to distance between a given male and a spawning female, or whether another male was already present. During casual observations, males were observed to feed in close proximity to a spawning female but not attempt to copulate with her. On less than 20 occasions, males were also observed to leave still-spawning females, and to have their place taken within minutes by another male. Males were seen to move past females just

starting to spawn, and approach and copulate with a female, farther away, that had almost completed her egg mass.

Males were observed, although infrequently ( $n = 10$ , all species), to attempt copulation with another male both in the field and in the tank. In each case, the male attempted to mate with a male that was engaged in copulation with a female. In the mixed-species collection maintained in the tank, males were also observed to mate with congeneric females: *S. gallus* and *S. raninus* males with a *S. costatus* female, *S. raninus* males with a *S. gallus* female, *S. gallus* males with a *S. raninus* female, and mixed-species combinations of males with any female (e.g., *S. gallus* and *S. raninus* males with an *S. gallus* female, but not necessarily with the conspecific male in the primary male role). None of the females spawned after those copulations, unless a conspecific male was involved. In the separate collection of *S. pugilis* and *S. alatus*, individuals mated at random with no apparent distinction between species, and females spawned fertile egg masses, regardless of the male involved.

Non-spawning females were observed in the field and in the tank to drag males around with them while engaged in copulation. The attachment of male to female was through the verge. One female *S. gigas* was found to have dragged her partner over 25 m in 24 hours, as shown by the track marks made in the substrate between observation periods.

## DISCUSSION

The male reproductive system does not vary in position or detail among the species of conch included in this study, and presumably does not vary within the genus. Okutani (1965) mislabelled the prostate as "osphradium" in his illustration of a male *S. listeri* T. Gray 1852, as indicated by its position and the vas deferens leading up to it (see Little 1965 for correct placement of the osphradium).

The microscopic structure of the verge tip shows that goblet cells line the outside walls of the papillae among ciliated epithelium but are not found in the section between the rows of papillae. The presence of the high number of goblet cells suggests that they are providing mucus for the purposes of lubrication and facilitation of sperm movement during transfer to the female (Hughes 1986). The papillae may also aid in the holding onto the copulatory organ of the female in which case the mucus provided by the goblet cells may increase adhesion.

The prostate gland is very simple in microscopic structure, and presumably its function is the secretion of energy rich fluids and mucopolysaccharides into the semen. There are no visible golgi complexes or rough endoplasmic reticulum, as described by West (1978) for other species of molluscs, and the secretory cells appear to be modified cuboidal epithelium derived from the mantle.

The role of apyrene sperm in conch appears to be nutritive, as biochemical study has shown them to be made up of granules of polysaccharides (Koike and Nishikawa 1980). Since these sperm completely break down within 2 hours of release from the vas deferens, there appears to be an inhibitory factor present in the vas deferens that keeps these sperm intact. This breakdown could be the means whereby apyrene sperm provide nutrients for the maintenance of eupyrene sperm after deposition and storage in the female.

Other hypotheses that could account for the production of apyrene sperm in conch are: (1) facilitation of eupyrene sperm movement in the male and female, (2) provision of nutrients for the female and/or zygotes, and (3) displacement/inactivation of

eupyrene sperm from previous matings, which could also cause delay of subsequent mating by the female if her receptaculum seminis is full (Silberglied et al. 1984). A nutritional role is the most likely aspect of apyrene sperm function based on their biochemistry; however, displacement of another male's sperm cannot be ruled out. By saturating the female reproductive tract with semen, a male could displace other sperm already there, as well as block use of stored sperm by creating a mechanical barrier to the movement of sperm in and out of the receptaculum seminis of the female. Inactivation of other stored sperm does not appear to be a possibility, as multiple sires of egg masses have been reported (Steiner and Siddall, unpubl. manus.).

Delaying subsequent mating of the female by filling her receptaculum seminis to capacity would not obviously benefit a male unless the female is unlikely to find another mate before spawning. Since the female can apparently hold a male in position for an extended period of time, she does not need to use any stored sperm at all, providing that sperm transfer is taking place. Hence, subsequent matings are not delayed unless the female does not encounter another mate before she spawns again. In most conch species, individuals tend to remain in loosely organized colonies, so mates are easy to find (Catterall and Poiner 1983, pers. obs.).

Prolonged copulation, with or without sperm transfer, is considered to be a strategy used by the male to protect his investment of time and energy in producing sperm (Parker 1974, Cordero 1990); consequently, prolonged copulation becomes a post-insemination guarding tactic (McLain 1989) that would benefit male strombids such that their sperm would not be displaced. However, this strategy may actually be reversed in strombids, such that the female prolongs copulation in order to obtain as much semen as she can from the male. The energy-rich nature of conch semen (Koike and Nishikawa 1980) supports this hypothesis.

A male that copulates with a female of another species would obviously not be contributing to the next generation, and neither would a female who accepts him; however, if the male is contributing nutrients to the female via the semen, then it is to her advantage to accept him. Also, because such semen is costly to produce for the male, he should supply semen only to those females that will use it (Dewsbury 1982), such that he should mate only with spawning females, as was the case in *S. pugilis*.

Chemosensory ability of strombids appears to be limited, in that homing in on a conch of the opposite sex is good, but the ability to distinguish among mates once physical contact is made is poor. Males were observed to attempt copulation with another male already engaged in copulation with a female. Males appear to be attracted by chemotaxis (Kuwamura et al. 1983), but presumably become confused when they physically contact a male in close proximity to a female. Interspecific copulation has been observed (A. Stoner, pers. comm., pers. obs.), showing that strombids do not distinguish well between mates on the basis of species.

The interspecific copulations observed show that verge shape is not a mechanism for prevention of cross-breeding as suggested by Abbott (1960) as males were observed to copulate with congeneric females both in the tank and in the field; A. Stoner (pers. comm.) also reported observing a *S. gigas* male copulating with a *S. costatus* female. The function of extra projections on the verge in some species, such as *S. gallus* and *S. pugilis*, is unknown. Changes in verge shape may have arisen as a genetic corollary to other morphological changes. The natural barrier to interspecific mating is most likely the tendency of conch to form aggregations of the same species, and their slightly different habitat prefer-

ences. Extreme size differences would also prohibit some species from intermating (e.g., *S. gigas* with *S. pugilis*).

From the female's perspective, her acceptance of a mate, in terms of using his sperm for fertilization of her eggs, may depend on the amount and/or quality of his semen. If so, acceptance of a congeneric male would be to her advantage as long as sperm transfer was taking place, and she could use the semen for nutritional purposes. However, as mates are rare for some species of conch and density is low in some areas, acceptance of mates may depend on who is available. Mating strategies in *S. gigas*, which is severely overfished, may have changed due to their very low density. Copulation with a non-spawning female, vs. copulation with a spawning female, is much more prevalent in *S. gigas* and *S. costatus* (Appeldoorn, in press), and *S. gallus*, and *S. raninus* (pers. obs.), indicating that females of these species are more apt to accept males when they are available. The latter 2 species are so rare in Puerto Rico that the first male that comes along is acceptable, regardless of his species.

Mating preferences of *S. pugilis* follow those found in *S. gigas* and *S. costatus* (Appeldoorn, in press) but are more extreme: essentially all matings were with spawning females (copulating pairs, where the female was not spawning, were found to be a much lesser degree than in the other species). Primary males often remained in position on the female for an extended period of time, usually until the female had stopped spawning (about 8 hours to lay an average-sized egg mass). Bradshaw-Hawkins (1982) hypothesizes that this extended copulation is "guarding" behavior, whereby the male attempts to prevent the female from copulating with another male(s). However, a simpler explanation may be in the time needed to complete sperm transfer. Furthermore, the sex that controls duration of verge attachment has yet to be determined. Primary males were securely attached to the female but not the secondary males, indicating that the copulatory mechanism employed by primary and secondary males was different. Further

research is needed to investigate the mechanisms, and how they might relate to who was controlling duration of copulation.

Multiple-male matings in *S. pugilis* were rare compared to single-male matings, but still observed frequently enough to suggest that there is probably a limited advantage to the secondary male even when other mates are available, as was the case in the *S. pugilis* population studied. Due to declining stock densities caused by overfishing *S. gigas* mates are harder to find in some areas so mating preferences and strategies will probably change in that species.

Some of the reproductive behaviors manifested by strombid males and females were not explicable based on known biology and anatomy from the literature. The reproductive anatomy of females needed to be elucidated in order to determine how simultaneous copulation and spawning could occur, how more than one male was able to mate with the same female at the same time, how a female was able to hold onto the primary male, but not the secondary, and how these phenomena would relate to mating preferences observed. Furthermore, the questions that have arisen during this research concerning mating strategies are partially answerable, but additional research is needed in order to clarify them. Conch have been shown to engage in multiple-male copulations. However, the advantage to the secondary male(s) is not clear. These males could be attempting to spread their investment around more than that allowed by females in order to hedge against unsuccessful reproduction (Halliday and Arnold 1987); however, the female may not necessarily be accepting his sperm, and he may be wasting his time.

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## REPRODUCTIVE ANATOMY AND BIOLOGY OF THE GENUS *STROMBUS* IN THE CARIBBEAN: II. FEMALES

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**ABSTRACT** The reproductive anatomy and function of female *Strombus* (6 species) in the Caribbean were studied micro- and macroscopically. Female reproductive organs are composed largely of signet cells, except for egg and sperm tracts which are lined with ciliated epithelium and goblet cells. Specific organs for sperm deposition (bursa copulatrix) and storage (receptaculum seminis) in females are identified and described. The structure and orientation of the uterus, bursa copulatrix, sperm tract, and receptaculum seminis suggest that copulation does not interfere with spawning and that sperm deposited during spawning could be used preferentially for fertilization. Only one verge can fit in the bursa copulatrix at one time; secure attachment may be by swelling of the bursa copulatrix tissues and interlocking of its lamellae with papillae of the everted accessory pad of the verge. Secondary males may attempt to deposit sperm over the open portion of the uterine terminus.

**KEY WORDS:** Gastropoda, Strombidae, anatomy, reproduction, biology

### INTRODUCTION

In the Strombidae, females individually encapsulate their eggs and then place them in a tube. This tube is laid as a long continuous strand on sandy substrates (see Eisawy and Sorial 1968 for a detailed egg-mass description). As the strand is extruded, sand particles adhere to the exterior of the tube, which is molded into a crescent-shaped mound by the back and forth movement of the female's foot. Spawning will take place in the absence of substrate, but the egg mass is then irregularly shaped (Bradshaw-Hawkins 1982, pers. obs.). The number and size of eggs per egg mass vary with species and individual, but the processes of egg-mass formation and deposition are the same (Robertson 1959, D'Asaro 1965, 1970, 1986).

Little (1965) described the general appearance of the soft parts of the queen conch, *Strombus gigas*, but only labelled the gonads, egg groove and uterus of the female; he specifically did not discuss the reproductive organs. Haller (1893) depicted the excised reproductive tract of a *Strombus* female but did not study function of any of the parts. Oogenesis and gonadal morphology in *S. gigas* were studied in detail by Egan (1985).

Females are known to store sperm, as they will spawn in the absence of males for several weeks after last copulation (D'Asaro 1965, Weil and Laughlin 1984). Although copulation has been observed, little is known of the actual process. The male inserts his verge under the lip of the female's shell and into her mantle cavity, but the shell and mantle block direct observation. Hence, the sites of sperm deposition and storage in the female were previously unknown. The mechanism of sperm transfer had never been studied nor had the processing of eggs through the reproductive tract. Bradshaw-Hawkins (1982) showed that fertilization of eggs takes place approximately 45 minutes after extrusion, with free sperm mixed in the matrix surrounding the egg capsules within the tube.

Female *Strombus* have been observed to mate with males while in the process of egg deposition, and more than one male can copulate with a female at the same time. In many species of

gastropods with internal fertilization, only one male can copulate with the female at any given time; the arrangement of internal reproductive organs of the female prevents more than one male from copulating (Fretter 1953, 1984). Also, in many species, females cannot deposit eggs while engaged in copulation due to blockage of the vagina by the male (Fretter and Graham 1962, 1964).

The purpose of this investigation was: 1) to accurately depict and describe the reproductive system of female *Strombus* at the macro- and microscopic levels, 2) to determine the sites of sperm deposition and storage, 3) to follow the path of the eggs through the reproductive tract as well as the changes that occur en route, and 4) to examine mechanisms that allow simultaneous spawning and copulation.

### METHODS

Female *S. gigas* ( $n > 100$ ), *S. costatus* ( $n > 100$ ), *S. raninus* ( $n = 2$ ), *S. gallus* ( $n = 3$ ), and *S. pugilis* ( $n > 100$ ) were collected off the southwest coast of Puerto Rico. Live female *S. alatus* ( $n = 28$ ) were obtained from southern Florida. Females were removed from their shells and the reproductive tracts dissected. Portions of the various reproductive tissues were excised from freshly opened, unrelaxed animals (2 females of each species) and fixed in Bouin's solution for 24 hours. Any females engaged in copulation and/or spawning were immediately dissected upon return to the laboratory. Females and males were also kept in a tank with flow-through seawater at the laboratory in order to reduce time between being observed copulating/spawning and dissection.

Females, of *S. gigas* and *S. costatus*, that were found spawning in the field or tank, were immediately dissected in order to watch eggs moving through the reproductive tract. Eggs were removed from various points along the tract in order to locate the area where encapsulation occurred. For both females spawning alone and those spawning and engaged in copulation, scrapings were taken from various portions of the tract and examined for the presence of sperm. Also, 40 immature *S. gigas* were dissected in order to look at reproductive organ development both before and after external genitalia began forming. Of these 40, 21 were female.

Sperm storage capacity was determined by mechanically emp-

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tying the receptaculum seminis and refilling it with water, using a calibrated syringe. Maximum storage capacity was defined as the volume injected until back pressure caused the water to run out. Purple dye was added to the water as a marker for leaks.

A mixed collection of adult *S. raninus*, *S. gallus*, and *S. costatus* males and females, as well as a separate collection of adult *S. pugilis*, and *S. alatus*, were maintained in a free-flowing saltwater tank in order to observe mating and spawning behaviors, and to obtain spawn from females. The rarer species, *S. raninus* and *S. gallus*, were specifically observed in order to verify that mating and spawning behavior was the same for all species of strombids in the Caribbean. Of specific interest were observations of interspecific copulations that would not have been possible in the field due to the paucity of individuals, depth and bottom-time restrictions when using SCUBA for research purposes, and that these species of conch are rarely found intermixed in the field.

Populations of *S. pugilis* were observed in the field as part of a study on reproductive seasonality and periodicity (Reed 1994). Mating/spawning pairs of the other species were similarly observed when chanced upon. All species were observed for type of copulatory mechanism used by the male (whether he was the primary or secondary male, see Part I), and for spawning, if female.

Fixed tissues for histological analyses were dehydrated in 95% ethanol and embedded in paraffin. Sections were cut 6–10  $\mu\text{m}$  in thickness and mounted on albuminized slides. Staining was with hematoxylin and eosin according to Harris' regressive method in Howard and Smith (1983). Slides were then examined using light microscopy.

An attempt was made to observe copulation directly by adapting the window method of Stauber (1940). A square window of shell was cut from the dorsal side of the last body whorl of 50 *S. pugilis* females, and replaced with a plexiglass window, cemented with silicon. Half of the females were returned to the field, and the others were kept in a tank with males from the same population. None of the experimental females ever engaged in copulation or spawned. In the field, these females were subject to predation due to ease of access through removal of the window. In both field and tank, experimental females tended to bury themselves and remain so for extended periods of time. The windows were covered over with a thin layer of shell within 7 days.

## RESULTS

### General Anatomy

Female anatomy did not differ among the species studied. The following description of the reproductive system thus applies to all species included in this study. Figure 1 illustrates a generalized adult female strombid with the mantle cavity opened from left to right. Various organs are labelled to show the relative orientation and position of reproductive structures.

The ovary overlies the digestive gland at the distal end of the visceral mass (located well up in the spire of the shell when alive). Ovarian ducts run throughout the ovary, eventually uniting to form a single fallopian tube that runs along the surface of the visceral mass before entering the uterus (Fig. 1). The ovary and associated ducts are separate from the digestive gland, and are covered by a thin layer of pallial epithelium, as is the rest of the visceral mass.

The fallopian tube enters the uterus posterior to the bursa copulatrix, the copulatory organ where the male inserts his verge (Fig. 2). This primary portion of the uterus is an enclosed groove that has turned back on itself several times, with adjacent walls fused

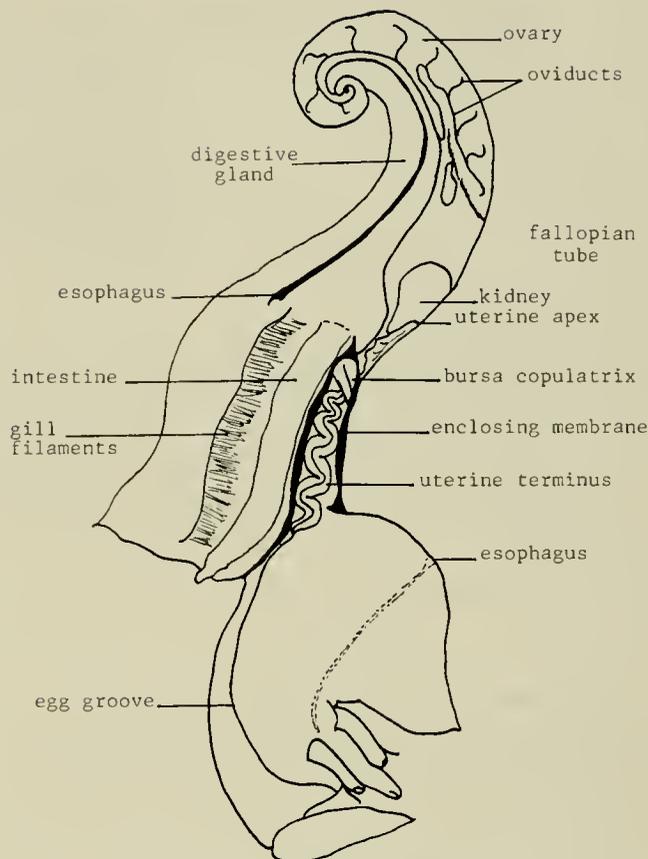


Figure 1. Schematic drawing of a generalized female *Strombus* with mantle cavity laid open to the right showing position and orientation of the reproductive organs.

together to form a ball-like shape (herein designated, "uterine ball").

From the uterine ball, a single enclosed groove runs dorsal to the lumen of the bursa copulatrix to a junction formed by mantle ligaments that hold the bursa copulatrix and uterus together, and from which the final portion of the uterus (herein designated "uterine terminus") leaves. At this junction, the single groove enters the main portion of the uterus. This portion of the uterus is formed by fusion of the walls of a single groove that has turned back on itself, and is enclosed by a membrane. The main portion of the uterus is shaped as an inverted U, first running posterior and parallel to the single groove portion of the primary uterus, then turning to run parallel to itself dorsal to the intestine. A prominent projection (termed "uterine apex") runs posterior from the fold of the "U." There are usually projections from the posteriorly running portion of the main uterus (herein called, "uterine arms"), which can number from 0 to 8, and be simple or branched. The uterine apex and any uterine arms are extensions of one of the grooves comprising the main portion of the uterus.

The uterine terminus is a single open groove flanked on both sides by membranes (herein designated, "enclosing membranes") formed from extensions of the mantle and mantle ligaments that hold the reproductive organs in place. The uterine terminus ends just short of the mantle, where a simple groove continues along the mantle and onto the foot, as a fold of the epithelium. The latter groove is the visible portion of the egg groove when the live animal is extended from the shell.

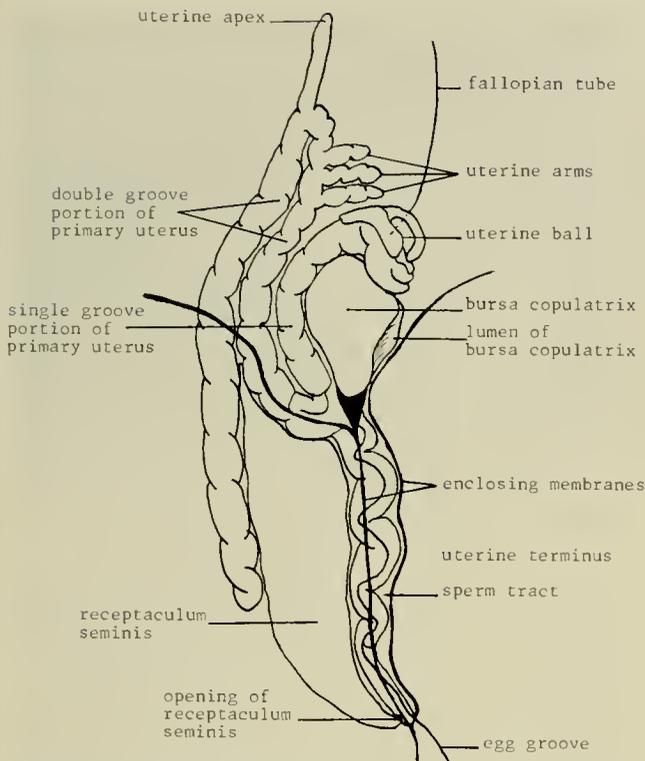


Figure 2. Schematic drawing of a generalized female *Strombus* reproductive tract removed from animal.

Eggs were never found in the oviducts unless the female was actually in the act of spawning. Eggs from the fallopian tube first enter the uterine ball, then move along in the single groove to the junction where they continue up one side of the double groove system to the uterine apex, entering any projections along the way. They continue down the main portion of the uterus, parallel to the intestine, then back up again to the uterine apex, along the other side of the double groove system, to the junction formed by the uterine terminus, single groove portion of the primary uterus, mantle ligaments, and enclosing membranes. The eggs continue along the uterine terminus to the egg groove, and are eventually deposited on the substrate.

Located between the uterine terminus and the enclosed double-groove portion of the uterus is the receptaculum seminis. The single opening to the sperm storage sac is located at the end of the uterine terminus, just posterior to the egg groove on the mantle. Sperm storage capacity depends on the size of the female. *S. gigas*, with a shell length of 27.5 cm, can store up to 5 mL of semen; *S. costatus*, with a shell length of 15.7 cm, 3 mL; *S. raninus* and *S. gallus*, with shell lengths of 91.2 cm and 109.2 cm, respectively, 1 mL; and *S. pugilis* and *S. alatus*, with shell lengths of 10.2 cm, 0.5 mL.

The bursa copulatrix is the site of sperm deposition during copulation. It is a separate organ from the uterus, with its opening oriented away from the uterus. A fold (termed "sperm tract") formed by the dorsal enclosing membrane and the wall of the uterine terminus runs from the bursa copulatrix to the receptaculum seminis opening. Semen is transported along this tract from the bursa copulatrix to the receptaculum seminis opening. Due to orientation of the bursa copulatrix, copulation should not interfere with spawning. Direct deposition of sperm into the receptaculum sem-

inis is not possible as the opening is too small to allow a verge to penetrate.

Figure 3 illustrates the relationship of the receptaculum seminis opening to the uterine terminus and enclosing membranes. The receptaculum seminis opening comes from underneath the uterine terminus, not within it. Sperm travels beside the outer wall of the uterine terminus, between the gland and enclosing membrane.

Examination of eggs taken from various portions of the reproductive tract revealed that encapsulation occurs in the uterine terminus. Sperm is also added to the egg matrix in the uterine terminus. No sperm were found in any of the other portions of the uterus, only in the uterine terminus. Capsules then enter the egg groove where the tube is formed around them.

The reproductive organs begin development at the same time as external genitalia (see Appeldoorn 1988 for external genitalia development in *S. gigas*). In immature females, the uterine terminus is a thin white line on the mantle wall. The rest of the uterus forms by extension of the uterine terminus. Immature females are distinguishable from immature males, before external genitalia begin development, by a slight bulge in the line where the bursa copulatrix will grow. When a female is reproductively active, the uterine tissues become engorged. The receptaculum seminis develops as an invagination of the mantle from the uterine terminus towards the intestine. Gonad tissue is white, thin, and smooth in appearance in immature females. As the female matures and becomes reproductively active, the gonad tissue takes on a grainy appearance as the developing follicles become visible to the unaided eye. Coloration may range from cream to tan. Eggs are found in the oviducts and fallopian tube only when the female is spawning.

### Histology

There were no discernible differences in the tissues examined between or among species; thus, results are applicable to any female conch of the species studied. Longitudinal and transverse sections through portions of the uterine grooves showed them to be completely composed of signet cells (Egan 1985). Ciliated epithelium interspersed with goblet cells lines the groove (Fig. 4). The membranes enclosing the uterus are composed of connective tissue and muscle fibers, overlaid by squamous epithelium. The egg groove (Fig. 5) is lined with ciliated columnar epithelium under

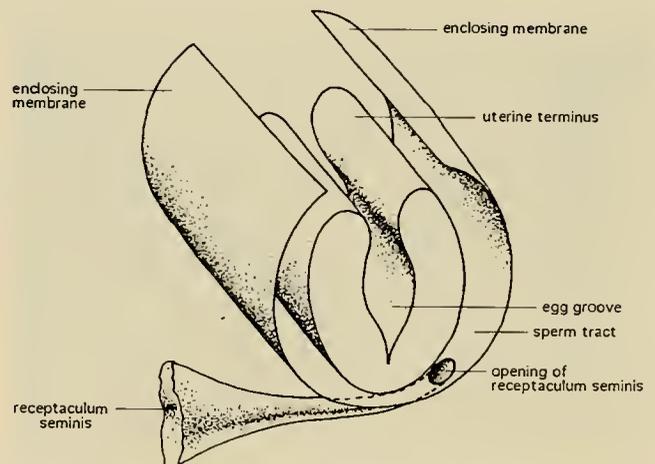


Figure 3. Schematic drawing of the cross-section through reproductive tract of female *Strombus* showing relationship of receptaculum seminis opening to uterine terminus and enclosing membranes.

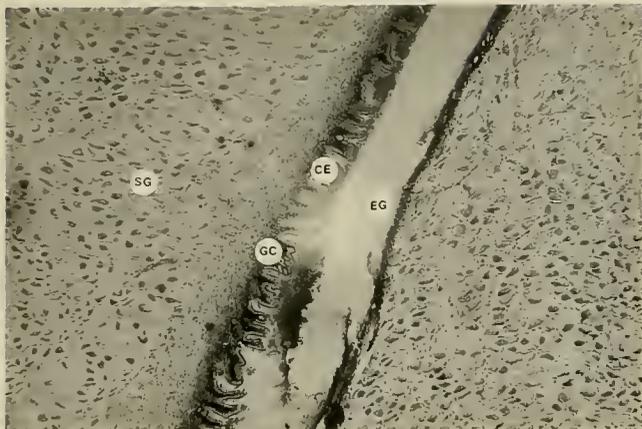


Figure 4. Longitudinal histological section through a portion of the uterus, using *Strombus gigas* as the example ( $\times 100$ ). CE, ciliated epithelium; EG, egg groove lumen; GC, goblet cell; SG, signet cells.

which a layer of longitudinally oriented muscle fibers runs. The groove itself is formed from the epithelium of the mantle and foot.

The bursa copulatrix is composed of signet cells interspersed with a few muscle fibers (Fig. 6). Numerous ducts lined with squamous epithelium run throughout the organ. Lymph vessels are also present, and are ringed with circular muscle. The lumen of the bursa copulatrix is lamellated with ciliated epithelium under which runs a layer of longitudinally orientated muscle fibers of varying thickness.

The walls of the receptaculum seminis (Fig. 7) are composed of muscle fibers running in longitudinal direction and interspersed with connective tissue. The interior of the sac is lamellated. The lamellae are covered with ciliated epithelium and goblet cells. There are a high number of leukocytes present in the tissue immediately surrounding the lumen. The lumen is partially filled with sperm matrix.

#### Behavioral Observations

Observations of the mixed-species collection of conch in the tank showed that females would engage in copulation with congeneric males. Attempts to remove the male showed that he was securely attached to the female via his verge. However, such fe-

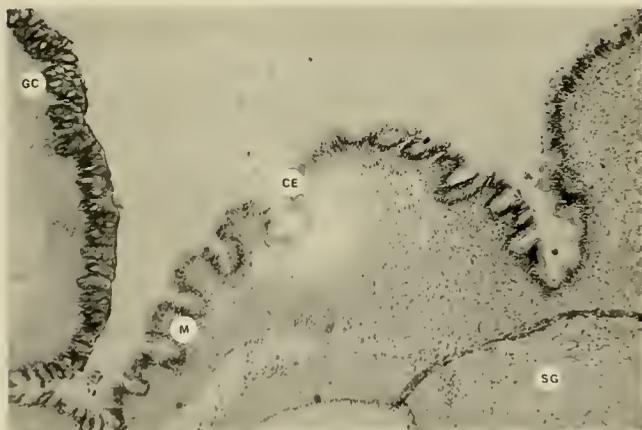


Figure 5. Transverse histological section through the egg groove on the mantle, using *Strombus pugilis* as the example ( $\times 100$ ). CE, ciliated epithelium; EG, egg groove lumen; GC, goblet cell; M, muscle; SG, signet cells.

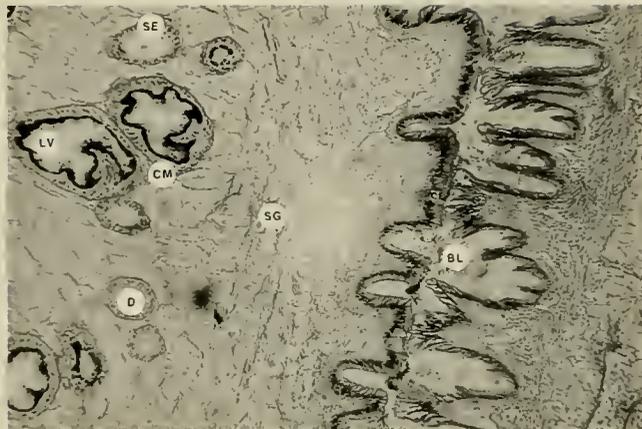


Figure 6. Longitudinal histological section through the bursa copulatrix, using *Strombus gigas* as the example ( $\times 100$ ). BL, bursa copulatrix lumen; CE, ciliated epithelium; CM, circular muscle; LV, lymph vessel; SE, squamous epithelium; SG, signet cells; D, ducts.

males did not spawn from those matings unless a conspecific male was involved (except in the separate collection of *S. pugilis* and *S. alatus*, where females spawned fertile egg masses, regardless of the male involved). Due to the rarity of specimens (only 6 *S. raninus* and 7 *S. gallus* were found in 6 years of study), such females were not dissected, so sperm transfer was not verified, only assumed to have taken place. Non-spawning females were observed to drag a male (regardless of species) around with them, while engaged in copulation. Females were seen to copulate and spawn simultaneously, as well as to have multiple partners, including one case of a female *S. costatus* engaged in copulation with a male *S. gallus* and a male *S. raninus*, although she did not spawn.

#### DISCUSSION

Female reproductive anatomy did not vary among the species studied and presumably is similar to other *Strombus* species.

Since the uterus is composed of signet cells, which are a type of connective tissue used for energy storage (Egan 1985), energy rich secretions are probably being added to the eggs in the enclosed uterine portions. Eggs may need the extra time represented



Figure 7. Transverse histological section through the receptaculum seminis, using *Strombus costatus* as the example ( $\times 100$ ). CE, ciliated epithelium; M, muscle; RL, receptaculum seminis lumen; SG, signet cells; SM, sperm matrix.

by their extended length of travel from the ovary via the uterus for additional maturation, as eggs were never found in the oviducts of any female unless she was actively spawning at time of capture. If so, energy from signet cells might aid in maturation.

The location of the opening of the receptaculum seminis potentially allows mixing of stored sperm with the eggs just before they enter the tube. Fresh sperm deposited in the bursa copulatrix will also be moving in the same direction as the eggs, and can easily be added to the matrix at the same time as stored sperm. Bradshaw-Hawkins (1982) showed that fertilization occurred approximately 45 min after extrusion of the egg strand of *S. pugilis*, and D'Asaro (1965) showed the same for *S. gigas*. Arrangement of the female reproductive glands does not allow freshly deposited sperm to enter the egg tract prior to the beginning of the uterine terminus. Movement of sperm and eggs is by ciliary action, which must be unidirectional in the egg tract prior to the uterine terminus in order to avoid blockage of the reproductive tract as there is no separate sperm tract except in the area of the uterine terminus. Also, there is no connection between the sperm tract and any portion of the uterus prior to the uterine terminus. Thus, stored sperm cannot come in contact with eggs prior to their arrival in the uterine terminus.

Where sperm is actually first mixed with the eggs is not known, as it could take place at the beginning of the uterine terminus or any point along its length to the receptaculum seminis opening. The general form of the uterine terminus and enclosing membranes suggest that the female can open or close the enclosing membranes around the uterine terminus to form a closed tube (Fig. 3). The orientation of muscle fibers in the enclosing membranes, as well as the ciliated epithelial lining of both enclosing membranes and uterine terminus support this idea. If so, then sperm could be forced from the sperm tract up and over the side of the uterine terminus and into the groove where the eggs are. Ciliary action within the sperm tract may be reversible such that sperm is brought from the receptaculum seminis opening up to the beginning of the uterine terminus. Also, whether sperm can penetrate the capsule of the egg is not known. If not, then sperm must be mixed with the eggs prior to encapsulation, at the beginning of the uterine terminus. If sperm can penetrate the capsule, then mixing could occur at any point along the uterine terminus.

The bursa copulatrix had not been previously described for *Strombus*. In previous accounts by Haller (1893) and Little (1965), the bursa copulatrix was misidentified as part of the uterus. The duct system revealed in the histological sections of the bursa copulatrix is probably used for inflating the tissue with water and/or lymph around the verge such that the verge is securely held. Also, lamellae in the lumen of the bursa copulatrix could interlock with the papillae in the tip of the verge creating more resistance to withdrawal by the male. Consequently, one disadvantage to the male using the bursa copulatrix could be the possibility of having his verge torn off, if the female decides to move. Although rare, males ( $n = 21$ ) were collected from the field who had lost their verges, and verge loss could be induced by pulling the male away from his partner until the verge broke.

The verge of the male must enter the bursa copulatrix on the side away from the uterus, so its presence would not incommode the female during spawning. Sperm would be transported along the outside of the uterine terminus to the receptaculum seminis without interfering with egg movement. This arrangement of organs has the important advantages of not only allowing the female to mate and spawn at the same time, but also guaranteeing a fresh

supply of sperm for fertilizing her eggs, as well as being able to store excess sperm for future use.

The relatively small size of the bursa copulatrix in comparison to the verge probably means that the bursa copulatrix can accommodate only one verge at a time, as is the case for other prosobranchs with internal fertilization (Fretter 1953). The open nature of the uterine terminus and its enclosing membrane would allow other males to deposit sperm in this area, which may explain observations of multiple males engaged in copulation with a single female, unless the female can exclude such males by closing the membranes around the uterine terminus. However, there is probably an advantage to using the bursa copulatrix, such as stimulation of sperm transport directly to the uterine terminus point where sperm and eggs mix and/or to the receptaculum seminis. Single copulations with non-spawning females in *S. pugilis* were so rare (only 4 cases observed in 2 years of observation) that double copulations with a non-spawning female would be absent by chance alone.

Whether mixing of sperm takes place during deposition by more than one male at the same time or within the receptaculum seminis is not known, and whether the female can preferentially use sperm from one male or another is also not known. In the absence of mixing, the single opening into the receptaculum seminis implies that the last sperm in is first sperm out.

There are many advantages hypothesized for multiple mating by females (Halliday and Arnold 1987), some of which may apply to strombids. The female may merely be taking advantage of the nutritious nature of the semen, which is composed of energy-rich molecules contained mostly within apyrene sperm (Koike and Nishikawa 1980). Other benefits that could apply to female strombids are: 1) hedging against male sterility, 2) receiving insufficient sperm from a depleted male, 3) guarding against genetic abnormalities/sperm inviability due to long-term storage, 4) producing genetically diverse progeny, and 5) promoting sperm competition. Hypotheses 2 and 3 are applicable to conchs as males do mate repeatedly within a short time period with different partners (pers. obs.), and females do store sperm on a long-term basis. However, all these supposed advantages could have arisen merely as a simple genetic corollary to selection on male mating tendency. If male mating tendency is shifted towards promiscuity by selection, then female mating tendency would also be expected to go in the same direction because of genetic linkage of the trait.

The relationship of the receptaculum seminis opening to the uterine terminus and enclosing membranes suggests that stored sperm can be mixed with the eggs at this point. Direction of ciliary movement within the sperm tract, however, could be opposite to that of the uterine terminus such that sperm is moved from the opening up to the beginning of the uterine terminus. This possibility cannot be ruled out without further study. The physical arrangement of the reproductive organs may be a result of phylogenetic constraint, rather than what is most efficient, due to the availability of tissues and their differentiation in the developing animal.

Multiple sires have been shown for *Strombus gigas* egg masses (Steiner and Siddall, unpubl. manus.), showing that females do accept sperm from more than one male. On the other hand, females have been observed to spawn completely or partially infertile egg masses (pers. obs., M. Davis, pers. comm., Weil and Laughlin 1984), indicating that a female cannot distinguish between viable and inviable sperm. Consequently, multiple mating may also be a strategy whereby the female hedges against such

occurrences (Halliday and Arnold 1987). Also, multiple mating by the female allows her to guard against receiving insufficient sperm from a depleted male, since strombid males have been observed to copulate with different females within a short time. The female would, as well, be guarding against male infertility. Although rare, one *S. gigas* male, that was later found to be infertile, did copulate with some females, who then spawned infertile egg masses.

Since eggs were never found in the oviducts of non-spawning females, copulation may be the stimulus required to initiate ovulation, and once ovulation begins, the female will spawn regardless of whether or not sperm transfer has taken place. This hy-

pothesis is supported by those females who did spawn infertile egg masses after copulation with the infertile male. If ovulation is triggered by copulation, the eggs may need the extra time, represented by their extended travel through the uterine system, for further maturation. This possibility could also explain why sperm is not mixed with eggs prior to their arrival in the uterine terminus.

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## TEMPORAL VARIABILITY IN NATURAL MORTALITY OF GREEN TIGER PRAWNS, *PENAEUS SEMISULCATUS*, IN KUWAIT WATERS

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**ABSTRACT** The temporal variability in natural mortality of green tiger prawns, *Penaeus semisulcatus*, in Kuwait waters from 1986 to 1989 was examined by using a variant of catch-curve model with the relative abundance data during the fishing closed seasons. The estimated natural mortality rates for these 4 years varied from 0.27 to 0.51, 0.17 to 0.33, and 0.23 to 0.42 month<sup>-1</sup>, respectively, for males, females and sexes combined. The natural mortality rates for female shrimp were always lower than those for males for all the 4 years studied although the differences were not significant in 1987, 1988, and 1989, and only marginally significant in 1986. It was found from the confidence intervals constructed by bootstrap and percentile methods that the temporal changes in natural mortality rates were not statistically significant ( $P > 0.05$ ) among 1986, 1987, and 1989, and between 1987 and 1988; the natural mortality rates in 1988, however, were significantly higher ( $P < 0.05$ ) than those in 1986 for males, females and sexes combined, and than those in 1989 for males and sexes combined. The high natural mortality rate in 1988 might be caused by the combination of high water temperature and low salinity. The temporal variations in natural mortality reflected the population dynamics and the environmental effects on the dynamics, and would practically affect the estimation of the population abundance and fishing mortality when applying a model incorporated with natural mortality.

**KEY WORDS:** Temporal variability, natural mortality, green tiger prawns

### INTRODUCTION

Natural mortality rate is a very difficult parameter to estimate. Because of the lack of information on the variability in natural mortality, if an estimate of natural mortality for a species is obtained by some source or by some method, it is always being considered constant and being repeatedly applied to estimate the population abundance and fishing mortality rate over years with stock assessment models such as cohort analysis (Gulland 1965, 1983, Pope 1972) and length-based cohort analysis (Jones 1981). For example, the natural mortality rate for North Sea plaice was estimated to be 0.1 with the data during 1939-1945 period when the fishery is almost closed because of World War II (Beverton and Holt 1993). This value has been applied by researchers in their studies because of the lack of a credible alternative, and almost all other estimates have been vulnerable to criticism on statistical grounds (Garrod 1988). North Atlantic cod is another reexample, of which an assumed natural mortality rate has been repeatedly used in stock assessment (Baird and Bishop 1986, 1989). It is apparent, however, that natural mortality will vary from year to year, especially in stocks near the margins of their environmental range, and because of variations in the relative abundance of predators and prey (Anon 1986, Garrod 1988). It is generally hard enough to obtain one estimate of natural mortality rate, and the idea of trying to measure changes in natural mortality is quite frightening, although many reasons make one believe that natural mortality might change over time (Hilborn and Walters 1992). Fortunately, a closed season for shrimp fishery in Kuwait waters which has been put in force since 1981 (Mathews 1985) provides us such an opportunity to estimate the natural mortality rate. Xu et al. (1995) developed a method to estimate the natural mortality using the relative abundance data obtained from research vessel survey during the season closure. This paper will apply this method to study the variability in natural mortality of green tiger prawns, *Penaeus*

*semisulcatus* De Haan, over years with the relative abundance data during the closed seasons of 1986 through 1989.

### MATERIALS AND METHODS

The relative abundance data, i.e., the catch per unit effort (CPUE), during the fishing closed seasons of 1986, 1987, 1988 and 1989 were collected by monthly research vessel surveys in Kuwait waters with *RV Bahith*, a stern trawler (679 tons in gross tonnage) of the Kuwait Institute for Scientific Research. Each monthly *RV* survey included 7 to 10 fixed stations in Kuwait waters with each tow lasting from 30 to 50 min. A 3 to 6 kg subsample of all shrimp capture was used to obtain species composition and length-frequency. Carapace length was measured to the nearest 1 mm. Because the recruitment stocks were not fully exploited by the research vessel survey during the closed season (Xu et al., 1995), only the relative abundance data of the adult stocks (Table 1) were used to estimate the natural mortality rates (month<sup>-1</sup>) of green tiger prawns in 1986, 1987, 1988 and 1989.

Kuwait waters were partitioned into 3 areas, Kuwait Bay, Middle Area and Southern Area, based on the species distribution and geographical considerations (Xu et al., 1995). The relative size ratios of the 3 areas (i.e., Kuwait Bay: Middle Area: Southern Area = 3: 4: 5), were used as a weighting factor when compiling the monthly relative abundance data from each sampled station.

A variant of the catch-curve model developed by Xu, et al. (1995) to estimate the natural mortality rate,  $M$ , with the relative abundance data during the season closure was applied in this study:

$$\text{Log}(CPUE_i) = \beta_0 + \beta_1 t + \epsilon_i \quad (1)$$

where  $CPUE_i$  is the relative abundance in month  $i$ ;  $t$  is relative age;  $\epsilon_i$  is assumed to be independent random variable with mean zero and constant variance;  $\beta_0$  and  $\beta_1$  are 2 parameters to be estimated

TABLE 1.

Relative abundance (CPUE) of adult stock of green tiger prawns in Kuwait waters during the fishing closed seasons of 1986 through 1989.

Year	Months	Relative Age (Month)	Relative Abundance (No./hr)		
			Male	Female	Combined
1986	April	0.00	22	19	41
	May	1.20	20	17	37
	July	3.27	9	11	20
1987	May	0.00	40	23	63
	June	0.97	35	21	56
	July	2.30	24	21	45
1988	August	3.10	13	9	22
	May	0.00	33	27	60
	June	1.27	20	18	38
	July	1.73	10	19	29
1989	August	2.90	8	10	18
	May	0.00	29	33	62
	June	0.67	21	30	51
	July	1.63	17	27	44
	August	2.63	14	17	31

by the method of least squares. The natural mortality rate,  $M$ , was then estimated by  $M = -\beta_1$ .

The confidence interval for the estimated natural mortality rate was constructed by bootstrap and percentile methods (Efron and Tibshirani 1986, Hall 1992). From the fitted model (1) by the method of least squares, the residuals,  $\epsilon_i$ , were estimated by:

$$\hat{\epsilon}_i = Y_i - \hat{\beta}_0 - \hat{\beta}_1 X_i \quad (2)$$

where  $1 \leq i \leq n$ . A set of the estimated residuals  $\{\hat{\epsilon}_1, \dots, \hat{\epsilon}_n\}$  were randomly resampled with replacement, and a new set of residuals  $\{\epsilon_1^*, \dots, \epsilon_n^*\}$  were generated. A new set of dependent variables  $\{Y_1^*, \dots, Y_n^*\}$  corresponding to  $\{x_1, \dots, x_n\}$  were obtained by:

$$Y_i^* = \hat{\beta}_0 + \hat{\beta}_1 x_i + \epsilon_i^* \quad (3)$$

Equation (1) was then fitted again with a new set of  $\{(x_1, Y_1^*), \dots, (x_n, Y_n^*)\}$  to obtain new estimate of natural mortality rate. We repeated this process 200 times to produce 200 estimates, and the confidence intervals were constructed by the percentile method.

Water samples were collected monthly from the surface and 1 m above the bottom at 2 fixed observatory stations in Kuwait Bay. Water temperatures were measured *in situ* using a hydrolab profiler, and salinities were measured with a Beckman Salinometer. The relationships between natural mortality and water temperature and salinity were examined by using correlation and regression analyses.

## RESULTS

The estimated natural mortality rates of green tiger prawns in 1986, 1987, 1988 and 1989 varied from 0.27 to 0.51, 0.17 to 0.33, and 0.23 to 0.42 month<sup>-1</sup>, respectively, for males, females and sexes combined (Table 2). It was found from the confidence intervals that the temporal variabilities in natural mortality rates were not statistically significant ( $P > 0.05$ ) among 1986, 1987, and 1989, and between 1987 and 1988; the natural mortality rates in 1988, however, were significantly higher ( $P < 0.05$ ) than those

TABLE 2.

The natural mortality rates of green tiger prawns in Kuwait waters estimated by the method developed by Xu et al. (in press).

Year	Sex	Natural mortality rate	CI
		(month <sup>-1</sup> )	(month <sup>-1</sup> )
1986	Male	0.29	0.21–0.37
	Female	0.17	0.14–0.20
	Combined	0.23	0.17–0.28
1987	Male	0.35	0.26–0.43
	Female	0.25	0.10–0.39
	Combined	0.31	0.19–0.41
1988	Male	0.51	0.37–0.68
	Female	0.33	0.25–0.41
	Combined	0.42	0.39–0.45
1989	Male	0.27	0.21–0.32
	Female	0.24	0.17–0.32
	Combined	0.25	0.22–0.29

CI indicates 95% percentile confidence interval constructed by the bootstrap and percentile methods.

in 1986 for males, females and sexes combined, and than those in 1989 for males and sexes combined.

Although the differences in the estimated natural mortality rates between female and male shrimp were not significant in 1987, 1988, and 1989, and only marginally significant in 1986, the natural mortality rates were always lower for female shrimp than those for males for all the 4 years studied. This finding is in line with the results of the previous studies on this species by Siddeek (1991) and Xu et al. (1995).

The monthly water temperature and salinity from May to August were calculated from the two sampling stations in Kuwait Bay (Table 3). The missing value of salinity in August 1987 was extrapolated from the mean of August 1986, 1988, and 1989. Analyses of variance indicated significant differences in salinity ( $P = 0.004$ ) but no statistical differences in water temperature ( $P = 0.859$ ) among years. The highest average water temperature from May to August was found in 1988 and the lowest in 1989. Duncan's multiple range test (Montgomery 1991) showed that the salinity in 1986 and 1987 were significantly higher than those in 1988 and 1989. Correlation analyses showed that water temperature had a positive effect ( $r = 0.597, 0.147$  and  $0.480$  for male, female and sex combined, respectively) and salinity had a negative effect ( $r = -0.460, -0.828$  and  $-0.586$  for male, female and sex combined, respectively) on the natural mortality of green tiger prawns, although the relations were not statistically significant. The relationships between the natural mortality rates (month<sup>-1</sup>) of male, female and sexes combined shrimp and the average water temperature and salinity of May through August were estimated by the method of least squares:

$$\begin{aligned} \text{Male} & M = -2.526 + 0.311 T - 0.144 S \\ \text{Female} & M = 1.242 + 0.118 T - 0.108 S \\ \text{Combined} & M = -0.953 + 0.225 T - 0.125 S \end{aligned}$$

where  $T$  (C) and  $S$  (‰) represent water temperature and salinity, respectively.  $F$  test showed that the regression equation were significant at  $P = 0.120, 0.064$ , and  $0.008$ , respectively. The adjusted multiple coefficients of correlation were estimated to be 0.957, 0.988, and 1.0, respectively, for male, female and sex combined.

TABLE 3.

The monthly water temperature and salinity observed from 2 fixed observatory stations in Kuwait Bay.

Month	1986		1987		1988		1989	
	T(C)	S(‰)	T(C)	S(‰)	T(C)	S(‰)	T(C)	S(‰)
May	25.55	38.87	22.08	37.73	25.83	37.77	25.75	36.71
Jun	25.65	40.06	26.43	39.69	25.85	38.96	23.95	39.02
Jul	28.93	40.99	29.53	40.38	28.63	39.13	28.40	39.24
Aug	30.58	41.09	32.50	40.39*	30.63	39.50	29.95	40.58
Average	27.68	40.25	27.64	39.55	27.74	38.84	27.01	38.89

\* Salinity in August 1987 was estimated from the average of August 1986, 1988, and 1989. T and S represent water temperature and salinity, respectively.

## DISCUSSION

The application of the method proposed by Xu et al. (1995) to this study gave satisfactory results of estimated natural mortality rates. One might be able to apply this method to other species to estimate natural mortality rate if there is a season closure for the fishery. The method can also be extended to estimate total mortality rate when population declines due to both natural death and fishing mortality. As mentioned by Xu et al. (1995), however, the precision in the estimated relative abundance (CPUE) from research vessel surveys will directly affect the precision in the estimated mortality. The relative abundance data estimated from the survey are not only affected by the random sampling errors, but might also be affected by animal behaviour such as migration, schooling or aggregation. Much higher relative abundances for adult stock in April of 1987, 1988 and 1989 (i.e., 151, 109, and 267 No./hr), than those in February and March during the fishing season (Xu and Mohammed 1995) indicated that the relative abundances were apparently overestimated in April of 1987, 1988 and 1989, and was not used in estimation of the natural mortality rates in this study. The high catch rate for the adult stock in April of these 3 years might have resulted from the shrimp aggregation or spawning migration that would increase the catchability. If one ignored this fact, the natural mortality rate for these years would be overestimated.

The fact that the juveniles of *Penaeus* species have very high natural mortality rate has been recognized by many studies (Edwards 1977, Laney 1981, Minello et al. 1989, Haywood and Staples 1993, O'Brien 1994). For example, the natural mortality rates of juvenile *Penaeus merguensis* were 0.23 to 0.94 wk<sup>-1</sup> (Haywood and Staples 1993) compared to 0.05 wk<sup>-1</sup> of the adult (Lucas et al. 1979). The natural mortality rates of the adult shrimp for some *Penaeus* species in Australian waters have been estimated from tagging experiments, e.g., 0.064–0.14 month<sup>-1</sup> for *Penaeus latisulcatus* (Penn 1976, 1981), 0.21 month<sup>-1</sup> for *P. merguensis* (Lucas et al. 1979), 0.31 month<sup>-1</sup> for *Penaeus longistylus* (Dredge 1990), and 0.55–0.57 month<sup>-1</sup> for *Penaeus plebejus* (Lucas 1974, Penn 1981). The obvious differences in the estimates among the species might be due to the species characteristics, or temporal and spacial variations assuming that these estimates were correct. The estimated natural mortality rates for green tiger prawns in Kuwait waters for sexes combined in 1986,

1987 and 1989 in this study (Table 2) and in 1993 by Xu et al. (1995) fell into the middle of the wide range among the species, and were very close to that of *P. merguensis* (Lucas et al. 1979) and that of *P. longistylus* (Dredge 1990). Much higher natural mortality rate for green tiger prawns in Kuwait waters in 1988 (Table 2), which was close to that of *P. plebejus* (Lucas 1974, Penn 1981), might be due to the combination of high water temperature and low salinity (Table 3). The finding of positive influence of water temperature on natural mortality is also supported by the widely used regression model of natural mortality on life history and water temperature by Pauly (1980). Among the years from 1982 through 1988, the lowest bottom oxygen (Joseph et al. 1991) and largest volume of runoff from Shatt Al-Arab River to Kuwait waters (Lee et al. 1990) were recorded in 1988. The entire Kuwait waters were influenced by the freshwater from Shatt Al-Arab River in 1988 (Lee et al. 1990). This would certainly reduce the salinity which is negatively correlated with natural mortality of green tiger prawns. In addition to the possible influence of the abiotic factors on natural mortality, predation, a biotic factor, is probably one of the most important factors which directly affect the natural mortality (Vetter 1988). Unfortunately, there is no information available on this aspect in this study.

Many studies have shown that stock assessment models such as VPA or cohort analysis and length-based cohort analysis were very sensitive to the variations in natural mortality rate (Pope 1972, Ulltang 1977, Sims 1984, Lai and Gallucci 1988, Xu and Chiu 1995). This study indicated that the temporal variability in natural mortality could arrive to a significant level. The temporal changes in natural mortality reflected the population dynamics and the environmental effects on the dynamics, and would practically affect the estimation of population abundance and fishing mortality rate when applying a stock assessment model incorporated with natural mortality.

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## TOXICITY OF AMMONIA, NITRITE, AND NITRATE TO JUVENILE AUSTRALIAN CRAYFISH, *CHERAX QUADRICARINATUS*

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**ABSTRACT** Survival rates and metabolic (oxygen consumption) rates were examined in juvenile Australian crayfish, *Cherax quadricarinatus*, exposed to different concentrations of ammonia, nitrite, or nitrate. The relative tolerances of these crayfish to ammonia, nitrite, and nitrate are similar to those reported for other fish and crustacean species. No mortalities were observed in crayfish exposed to concentrations up to 25 mg/L total  $\text{NH}_3\text{-N}$  (0.54 mg/L un-ionized  $\text{NH}_3\text{-N}$ ). Crayfish exposed to 50, 100, and 200 mg/L total  $\text{NH}_3\text{-N}$  (1.07, 2.14, and 4.28 mg/L un-ionized  $\text{NH}_3\text{-N}$ ) survived an average of 40, 36, and 14 hr, respectively. Calculated  $\text{LC}_{50}$  values for 24, 48, and 96 hr were  $94.3 \pm 0.24$ ,  $76.3 \pm 0.13$ , and  $45.9 \pm 0.25$  mg/L total  $\text{NH}_3\text{-N}$ , respectively (2.02, 1.63, and 0.98 mg/L un-ionized  $\text{NH}_3\text{-N}$ ). No mortalities were observed in crayfish exposed to concentrations up to 10 mg/L  $\text{NO}_2\text{-N}$ . Crayfish exposed to 25, 50, and 100 mg/L  $\text{NO}_2\text{-N}$  survived an average of 96, 22, and 5 hr, respectively. Calculated  $\text{LC}_{50}$  values for 24, 48, and 96 hr were  $42.9 \pm 0.22$ ,  $37.1 \pm 0.16$ , and  $25.9 \pm 0.35$  mg/L  $\text{NO}_2\text{-N}$ , respectively. No mortalities were observed in crayfish exposed to nitrate concentrations up to 1000 mg/L. Oxygen consumption rates of crayfish exposed to fresh water (controls) were regulated over the  $p_{\text{O}_2}$  range of 20 to 5 kPa. Oxygen consumption rates of crayfish exposed to ammonia or nitrate did not differ significantly from those of control crayfish over the same range of environmental  $p_{\text{O}_2}$ . Oxygen consumption rates of crayfish decreased immediately upon exposure to nitrate and were never recovered. At 10 kPa  $p_{\text{O}_2}$ , oxygen consumption rates of crayfish exposed to nitrite were approximately 50% of controls. In conditions of low environmental  $p_{\text{O}_2}$ , nitrite could substantially affect the survival of individuals.

**KEY WORDS:** Crayfish, ammonia, oxygen consumption, toxicity

### INTRODUCTION

Ammonia ( $\text{NH}_3$ ) comprises 40 to 90% of the nitrogenous excretion of crustaceans (Parry 1960). Under aerobic conditions, ammonia can be oxidized by nitrifying bacteria (*Nitrosomonas* and *Nitrobacter* spp) to nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) (Sharma and Ahlert 1977). Ammonia and nitrite are the most common pollutants found in intensively managed aquaculture systems and, as concentrations increase, they can negatively affect the production of fish, crustaceans, and other aquatic organisms (Colt and Armstrong 1981). Nitrate can also increase in aquatic systems to relatively high concentrations; however, it does not appear to directly affect the health of individuals (Russo 1985).

For many of the commercial fish species, the lethal concentrations of ammonia, nitrite, or nitrate, including their potential mechanism(s) of toxicity, have been examined (Russo 1985, for review). Researchers suggest that acute exposure to high concentrations of ammonia directly affects the central nervous system of fish resulting in increased gill ventilation, hyperexcitability, convulsions, and other physiological aberrations which eventually lead to the demise of the animal. Researchers suggest that acute exposure to moderate concentrations of nitrite directly affects the health of fish by reducing the oxygen binding affinity of the respiratory pigment hemoglobin, resulting in a physiological hypoxia (Brockway 1950, Russo 1985). No direct effects of nitrate on the health of fish have been demonstrated. Excessively high nitrate concentrations, however, have been demonstrated to result in excessive algal blooms and eutrophication in ponds resulting in hypoxic environmental conditions that can be detrimental to fish.

Concentrations of ammonia and nitrite lethal to several crustacean species have also been determined. Knowledge of the potential mechanism(s) of toxicity of these compounds to crustaceans remains unclear. Despite conclusive evidence, some investigators suggest that ammonia and nitrite affect crustaceans in a manner similar to that observed in fish; by either affecting central nervous system functions or by binding to the respiratory pigment hemocyanin resulting in decreased oxygen binding affinity, respectively (Sanders et al 1992, Needham 1961). Few, if any, investigators have examined the lethal concentrations of nitrate or its potential mechanism(s) of toxicity to crustaceans.

Australian freshwater crayfish of the genus *Cherax* are rapidly gaining attention among commercial culturists worldwide. Crayfish in this genera are typically fast growers, attain large sizes (up to several kg), and have multiple spawns annually (Semple et al. 1995). One species, *Cherax quadricarinatus*, is gaining popularity among culturists in the southern latitudes of the US. Little information exists on the effects of water-born toxicants, such as ammonia, nitrite, or nitrate, on this culture species. Such information, however, is prerequisite to defining those conditions necessary for successful commercial production. The objectives of this study were to (1) determine acute tolerances for juvenile *C. quadricarinatus* exposed to different concentrations of ammonia, nitrite, or nitrate, and (2) estimate metabolic rates of juveniles acutely exposed to sub-lethal concentrations of ammonia, nitrite, and nitrate.

### MATERIALS AND METHODS

#### Animals

Juvenile *C. quadricarinatus* were obtained from broodstock females maintained in Campbell Hall at the University of Alabama

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at Birmingham (UAB). Broodstock individuals were held in raceways (28°C) with associated recirculating biofilters to ensure water quality and the health of broodstock animals. Juveniles used for experiments ranged in size from 9 to 13 mm total length (10 to 25 mg wet weight) and were fed AB crayfish feed (UAB Research Foundation). This diet has previously been demonstrated to support weight gain and survival of juvenile *C. quadricarinatus* (Meade and Watts, in press). Juveniles lacking appendages (claws or walking legs) were excluded for use in experiments.

#### Toxicity Assays

Preliminary experiments determined the range of concentrations of ammonia, nitrite, or nitrate to be examined (data not shown). For each test solution examined, 10 siblings were placed individually in polystyrene bowls containing 100 mL of the desired test solution. A minimum of 2 groups of siblings was used for all test solutions examined. In many cases, the variability in survival of siblings among females was high, thus numerous other groups of siblings from other females were exposed to the toxicants to increase the statistical value of mean lethal times and concentrations. All juveniles used for toxicity experiments were not fed during exposure to ammonia, nitrite, or nitrate.

Stock solutions of the toxicants were made by mixing 3.82 g reagent grade ammonium chloride, 4.92 g sodium nitrite, and 6.07 g sodium nitrate each with 1 l of conditioned freshwater (28°C, pH  $7.5 \pm 0.2$ , alkalinity  $70 \pm 5$  mg/L, hardness  $300 \pm 10$  mg/L, and chloride 450 mg/L). Final concentrations of the stock solutions were 1000 mg/L total nitrogen in the form of  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ , and  $\text{NO}_3\text{-N}$ , respectively. Serial dilutions of the stock solutions were made to attain the desired concentrations for the test solutions. Test toxicant solutions were all maintained at 28°C and, if necessary, were adjusted to pH  $7.5 \pm 0.2$  using the appropriate amounts of 1 M NaOH or HCl. Other water quality parameters of the test toxicant solutions (alkalinity, hardness, and chloride) were maintained at levels similar to stock solutions. Survival of juveniles was determined at concentrations of 0 (control), 25, 50, 100, and 200 mg/L total ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ); 0, 10, 25, 50, and 100 mg/L total nitrite-nitrogen ( $\text{NO}_2\text{-N}$ ); or 0, 10, 100, and 1000 mg/L total nitrate nitrogen ( $\text{NO}_3\text{-N}$ ). To determine  $\text{LT}_{50}\text{s}$  and  $\text{LC}_{50}\text{s}$ , juveniles were examined every hour for mortalities through the first 6 hr of exposure and every 6 hr thereafter for up to 120 hr (5 days). Death of the juveniles was determined by an apparent lack of movement when prodded with a blunt glass probe and by microscopic examination for a heartbeat. For all treatments, test solutions were replaced daily using static water renewal methods (Buikema et al. 1982). The dose response of juvenile crayfish was determined by plotting the probit of mortality transformed from percent mortality against log concentration (Buikema et al. 1982). Moving averages and interpolation were used to determine  $\text{LC}_{50}$  ( $\pm\text{SD}$ ) (Buikema et al. 1982). Un-ionized  $\text{NH}_3\text{-N}$  was determined using aqueous ammonia equilibrium calculations (Emerson 1975).

#### Respiratory Measurements

To standardize for metabolic condition, respiratory rates of all juvenile crayfish were measured 2 days following a molt. Oxygen consumption rates were measured using a closed-chamber, temperature-controlled (within  $\pm 0.5^\circ\text{C}$  of desired temp.) polarographic respirometer (Oxygraph 67097, Cyclobios-Paar, Austria) using methods described previously (Meade et al. 1994). Briefly,

sibling crayfish ( $n = 5/\text{treatment}$ ) were placed in a closed respirometer (28°C) and allowed to consume oxygen at tensions from fully air saturated conditions ( $\sim 21$  kPa) down to hypoxic conditions ( $\sim 1$  to 2 kPa) without the presence of ammonia or its oxidized derivatives. Juveniles were allowed to consume oxygen throughout this range of oxygen tensions in 2 trials to determine basal rates before exposure to ammonia, nitrite, or nitrate. Different concentrations of ammonia, nitrite, or nitrate were then introduced into the system and rates of oxygen consumption were again measured from air saturated to hypoxic conditions. Control crayfish were exposed to introductions of equivalent volumes of fresh water. Juveniles were exposed to concentrations of 0, 50, 100, 250, and 500 mg/L total  $\text{NH}_3\text{-N}$ ; 0, 25, 50, and 100 mg/L total  $\text{NO}_2\text{-N}$ ; and 0, 100, 250, 500, and 1000 mg/L total  $\text{NO}_3\text{-N}$ . The pH, alkalinity, hardness, and chloride concentrations of the experimental solutions were similar to those conditions of the solutions used in the previous toxicity assays. Oxygen consumption rates were recorded and analyzed using Datgraf Analysis software v 2.1 (M. Reck and R. Kaufmann, Innsbruck, Austria). Oxygen consumption rates in this study are abbreviated as  $n_{\text{O}_2}$  and reported as  $\text{pmol O}_2/(\text{s} * \text{mg wet weight})$ . Statistical comparisons of oxygen consumption rates were made using the unpaired t-test (Daniel 1987). Alpha for statistical analysis was set at 0.05.

## RESULTS

#### Toxicity Assays

No mortalities were observed in control individual throughout the experiments. Juvenile crayfish survived long durations when exposed to relatively moderate concentrations of ammonia (Table 1). At concentrations of total  $\text{NH}_3\text{-N}$  from 0 to 25 mg/L (0.54 mg/L total un-ionized  $\text{NH}_3\text{-N}$ ), no mortalities were observed through 120 hr of exposure. At higher concentrations, survival of different groups of siblings was highly variable; average  $\text{LT}_{50}\text{s}$  for 50, 100 and 200 mg/L total  $\text{NH}_3\text{-N}$  (1.07, 2.14, and 4.28 mg/L total un-ionized  $\text{NH}_3\text{-N}$ ) were 38, 19, and 13 hr, respectively. The calculated  $\text{LC}_{50}$  values for 24, 48, and 96 hr were  $94.3 \pm 0.24$ ,  $76.3 \pm 0.12$ , and  $45.9 \pm 0.25$  mg/L total  $\text{NH}_3\text{-N}$  (2.02, 1.63, and 0.98 mg/L un-ionized  $\text{NH}_3\text{-N}$ ), respectively. Survival was substantially reduced when juvenile crayfish were exposed to nitrite (Table 2). At 0 and 10 mg/L total  $\text{NO}_2\text{-N}$ , no mortalities were observed through 120 hr of exposure. At higher concentrations, survival was variable among groups of siblings, but not as variable as observed for crayfish exposed to ammonia. At 25, 50, and 100 mg/L total  $\text{NO}_2\text{-N}$ , average  $\text{LT}_{50}\text{s}$  were 96, 22, and 5 hr, respectively. The calculated  $\text{LC}_{50}$  values for 24, 48, and 96 hr were  $42.9 \pm 0.22$ ,  $37.1 \pm 0.16$  and  $25.9 \pm 0.35$  mg/L  $\text{NO}_2\text{-N}$ , respectively. No mortalities were observed in juvenile crayfish exposed to concentrations from 0 to 1000 mg/L total  $\text{NO}_3\text{-N}$  during the 120 hr exposure period (Table 3).

#### Respiratory Measurements

Oxygen consumption rates varied, averaging between 3.5 and 5  $\text{pmol O}_2/\text{sec} * \text{mg wet weight}$ , among individual juvenile *C. quadricarinatus* exposed to fresh water. Oxygen consumption rates were regulated over the  $p_{\text{O}_2}$  range from 18 to 4 kPa (Figs. 1, 2, and 3). Below about 4 kPa, oxygen consumption rates conformed to declining  $p_{\text{O}_2}$  until reaching zero. In most cases, once placed in the respiratory chamber, individual crayfish consumed

TABLE 1.

Toxicity of ammonia (NH<sub>3</sub>-N) to juvenile Australian crayfish, *Cherax quadricarinatus*.

Female #	Toxicity Level			Mean LT <sub>50</sub>
	Total	(Un-ionized)	LT <sub>50</sub>	
I	25 mg/L NH <sub>3</sub> -N	(0.54 mg/L)	>120 hr	
II	25 mg/L NH <sub>3</sub> -N		>120 hr	
III	25 mg/L NH <sub>3</sub> -N		>120 hr	
V	25 mg/L NH <sub>3</sub> -N		>120 hr	>120 hr
I	50 mg/L NH <sub>3</sub> -N	(1.07 mg/L)	48 hr	
II	50 mg/L NH <sub>3</sub> -N		48 hr	
III	50 mg/L NH <sub>3</sub> -N		24 hr	
IV	50 mg/L NH <sub>3</sub> -N		48 hr	
VII	50 mg/L NH <sub>3</sub> -N		30 hr	
VIII	50 mg/L NH <sub>3</sub> -N		24 hr	
IX	50 mg/L NH <sub>3</sub> -N		42 hr	40 hr
I	100 mg/L NH <sub>3</sub> -N	(2.14 mg/L)	48 hr	
II	100 mg/L NH <sub>3</sub> -N		12 hr	
III	100 mg/L NH <sub>3</sub> -N		24 hr	
V	100 mg/L NH <sub>3</sub> -N		18 hr	
VI	100 mg/L NH <sub>3</sub> -N		6 hr	
X	100 mg/L NH <sub>3</sub> -N		6 hr	28 hr
I	200 mg/L NH <sub>3</sub> -N	(4.28 mg/L)	24 hr	
III	200 mg/L NH <sub>3</sub> -N		12 hr	
IV	200 mg/L NH <sub>3</sub> -N		5 hr	14 hr

<sup>a</sup> Mean values only for siblings exposed to all levels of ammonia. Note: controls (0 mg/L NH<sub>3</sub>-N) in all experiments had no mortalities.

and reduced oxygen tensions to hypoxic conditions within 30 to 45 min. When acutely exposed to ammonia at concentrations up to 1000 mg/L (total NH<sub>3</sub>-N), no significant differences were observed in oxygen consumption rates between experimental and control individuals (Fig. 1). Observed initial oxygen consumption rates were slightly higher in individuals exposed to ammonia. Oxygen consumption rates returned to levels similar to those of control individuals after approximately 5 min. Thereafter, crayfish exposed to ammonia regulated oxygen consumption rates until conforming to declining oxygen tensions at about 4 kPa p<sub>O<sub>2</sub></sub>.

When acutely exposed to nitrite at concentrations up to 50 mg/L (total NO<sub>2</sub>-N), no significant differences were observed in oxygen consumption rates between experimental and control in-

TABLE 2.

Toxicity of nitrite (NO<sub>2</sub>-N) to juvenile Australian crayfish, *Cherax quadricarinatus*.

Female	Toxicity Level	LT <sub>50</sub>	Mean LT <sub>50</sub>
I	10 mg/L NO <sub>2</sub> -N	>120 hr	
II	10 mg/L NO <sub>2</sub> -N	>120 hr	>120 hr
I	25 mg/L NO <sub>2</sub> -N	96 hr	
II	25 mg/L NO <sub>2</sub> -N	96 hr	96 hr
I	50 mg/L NO <sub>2</sub> -N	24 hr	
II	50 mg/L NO <sub>2</sub> -N	24 hr	24 hr
I	100 mg/L NO <sub>2</sub> -N	6 hr	
II	100 mg/L NO <sub>2</sub> -N	4 hr	5 hr

Note: controls (0 mg/L NO<sub>2</sub>-N) in all experiments had no mortalities.

TABLE 3.

Toxicity of nitrate (NO<sub>3</sub>-N) to juvenile Australian crayfish, *Cherax quadricarinatus*.

Female #	Toxicity Level	LT <sub>50</sub>	Mean LT <sub>50</sub>
I	10 mg/L NO <sub>3</sub> -N	>120 hr	
II	10 mg/L NO <sub>3</sub> -N	>120 hr	>120 hr
I	100 mg/L NO <sub>3</sub> -N	>120 hr	
II	100 mg/L NO <sub>3</sub> -N	>120 hr	>120 hr
I	1000 mg/L NO <sub>3</sub> -N	>120 hr	
II	1000 mg/L NO <sub>3</sub> -N	>120 hr	>120 hr

Note: controls (0 mg/L NO<sub>3</sub>-N) in all experiments had no mortalities.

dividuals (data not shown). At a concentration of 100 mg/L total NO<sub>2</sub>-N, oxygen consumption rates in the crayfish began to decrease immediately (Fig. 2). When oxygen tensions decreased to 50% of air saturated conditions within the chamber (~10 kPa p<sub>O<sub>2</sub></sub>), oxygen consumption rates of the nitrite exposed crayfish were approximately 50% of the rates of the controls (p < 0.05). When oxygen tensions decreased to 30% of air saturated conditions (~6 kPa p<sub>O<sub>2</sub></sub>), crayfish exposed to nitrite appeared unable to consume oxygen (rates near zero).

When exposed to nitrate at concentrations up to 1000 mg/L (total NO<sub>3</sub>-N), no significant differences were observed in oxygen consumption rates between experimental and control individuals

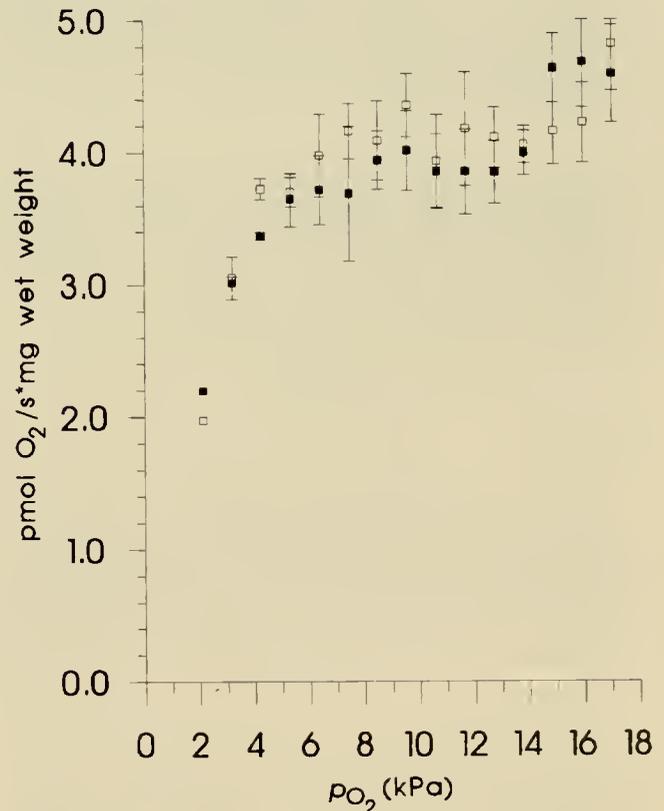


Figure 1. Oxygen consumption rates of juvenile Australian crayfish at various environmental p<sub>O<sub>2</sub></sub> when exposed to 0 mg/L (open squares) and 500 mg/L total NH<sub>3</sub>-N (10.7 mg/L un-ionized NH<sub>3</sub>-N) (closed squares). Each data point represents the mean ± SEM for 5 crayfish.

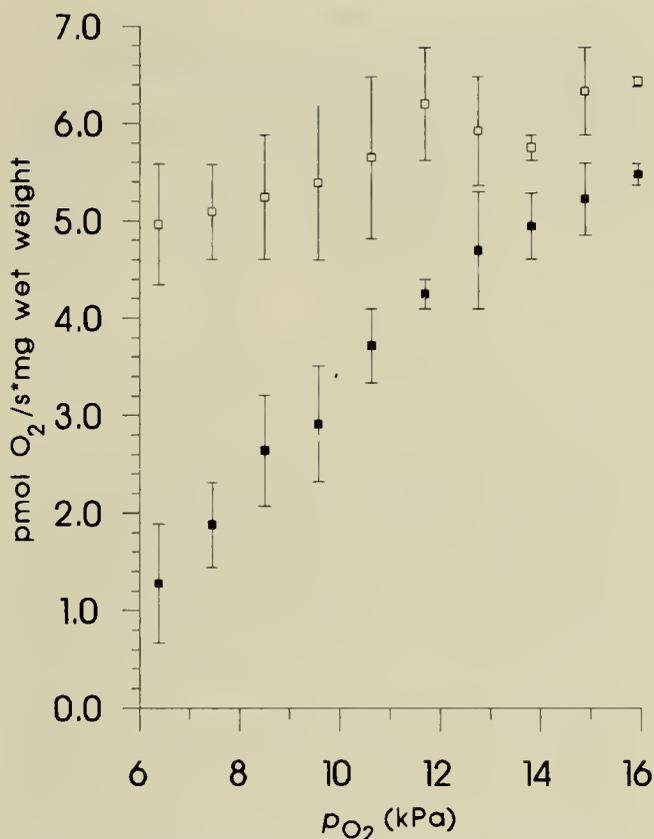


Figure 2. Oxygen consumption rates of juvenile Australian crayfish at various environmental  $p_{O_2}$  when exposed to 0 mg/L (open squares) and 100 mg/L  $NO_2-N$  (closed squares). Each data point represents the mean  $\pm$  SEM for 5 crayfish.

(Fig. 3). As in the ammonia treatments, initial oxygen consumption rates were slightly higher than those of controls. Oxygen consumption rates also returned to levels similar to those of control individuals after approximately 5 min. Thereafter, crayfish exposed to nitrate regulated oxygen consumption rates until conforming to declining oxygen tensions at about 4 kPa  $p_{O_2}$ .

## DISCUSSION

### Toxicity Assays

Many fish and crustacean species exhibit similar tolerances to ammonia. For example, 96 hr  $LC_{50}$  values of approximately 1 mg/L un-ionized  $NH_3-N$  have been reported for rainbow trout (*Salmo gairdneri*), largemouth bass (*Micropterus salmoides*), and larval tiger prawn (*Penaeus monodon*) (Thurston et al. 1981a, b, Roseboom and Richey 1977, Chin and Chen 1987). Variability in tolerance to ammonia among the different sibling groups in this study was high and was attributed to genetic variability. Nevertheless, juvenile crayfish, *C. quadricarinatus*, exhibited a tolerance to ammonia similar to that observed for other crustacean species (calculated 96 hr  $LC_{50}$  values of approximately 0.98 mg/L un-ionized  $NH_3-N$ ). Slightly higher ammonia tolerance, however, has been reported for other crayfish species. Evans (1979) reported 96 hr  $LC_{50}$  values between 3.2 and 3.8 mg/L total un-ionized  $NH_3-N$  for the adult crayfish, *Orconectes nais*. Liu et al. (1994)

also reported 96 hr  $LC_{50}$  values of approximately 3 mg/L total un-ionized  $NH_3-N$  for juvenile (0.7 to 0.9 g) *C. quadricarinatus*. Since water conditions such as pH, temperature, dissolved oxygen, and dissolved inorganics can affect the toxicity of ammonia (Russo 1985, for review), differences in experimental conditions can make comparisons among toxicity studies difficult. In this study, pH and temperature were maintained at similar values throughout the range of toxicant concentrations tested, however, dissolved oxygen concentrations were not monitored. Despite precise measurements, calculations of dissolved oxygen concentrations within the test containers (based upon water surface to volume ratios) indicated that oxygen should not have been a limiting factor affecting crayfish survival. We therefore suggest that minimal concentrations of un-ionized ammonia can cause significant mortalities of recently hatched juvenile *C. quadricarinatus*.

Nitrite toxicity to fish and crustaceans is highly variable from species to species. For example, 96 hr  $LC_{50}$  values of approximately 0.25, 1.25, and >67 mg/L total  $NO_2-N$  have been reported for rainbow trout (*S. gairdneri*), channel catfish (*Ictalurus punctatus*), and mottled sculpin (*Cottus bairdi*), respectively (Russo et al. 1981; Colt and Tchobanoglous 1976, Russo and Thurston 1977). For crayfish, 96 hr  $LC_{50}$  values of approximately 30 and 6 mg/L total  $NO_2-N$  have been reported for juvenile *Procambarus clarkii* and adult *P. simulans*, respectively (Gutzmer and Tomasso 1985, Beiting and Huey 1981). Lui et al. (1994) reported 96 hr  $LC_{50}$  values for juvenile *C. quadricarinatus* of approximately 5 mg/L total  $NO_2-N$  at a temperature of  $24 \pm 1^\circ C$  and a pH of  $8.6 \pm 0.1$ . In this study, 96 hr  $LC_{50}$  values of 25.9 mg/L total  $NO_2-N$  were observed for juvenile *C. quadricarinatus*. Water conditions have been observed to also affect the toxicity of nitrite, similar to the effects on ammonia toxicity (Russo 1985), thus making comparisons among toxicity studies difficult. Chloride, in particular, at concentrations greater than 100 mg/L, has been demonstrated to increase the resistance of crayfish to nitrite (Gutzmer and Tomasso 1985). Crayfish in this study may have demonstrated higher tolerance to nitrite considering chloride concentrations of the test solutions were relatively high (>400 mg/L). Nevertheless, we suggest that nitrite, in moderate concentrations, can cause significant mortalities of juvenile *C. quadricarinatus*.

Nitrate is considerably less toxic to aquatic organisms compared to ammonia and nitrite. Lethal concentrations (96 hr  $LC_{50}$ s) in excess of 500 mg/L total  $NO_3-N$  have been reported for Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*S. gairdneri*), and channel catfish (*I. punctatus*) (Russo 1985, for review). In this study, juvenile Australian crayfish survived long durations (>120 hr) with no mortalities when exposed to total  $NO_3-N$  concentrations of 1000 mg/L. Nitrate concentrations approaching levels of 500 mg/L total  $NO_3-N$  have been reported in large, recirculating artificial seawater systems (Pierce et al. 1993); however, concentrations in fish culture systems are seldom greater than 70 mg/L (Knepp and Arkin 1973). Based upon the results of this study, it is unlikely that nitrate will directly affect the health of intensively cultured juvenile *C. quadricarinatus*.

### Respiratory Measurements

The mechanism of toxicity of ammonia and nitrite to fish and crustaceans is not fully understood. Several investigators have suggested that ammonia and/or nitrite may affect the health of fish and crustaceans directly by altering normal respiratory functions (Brockway 1950, Needham 1961, Kiese 1974, Sanders 1992).

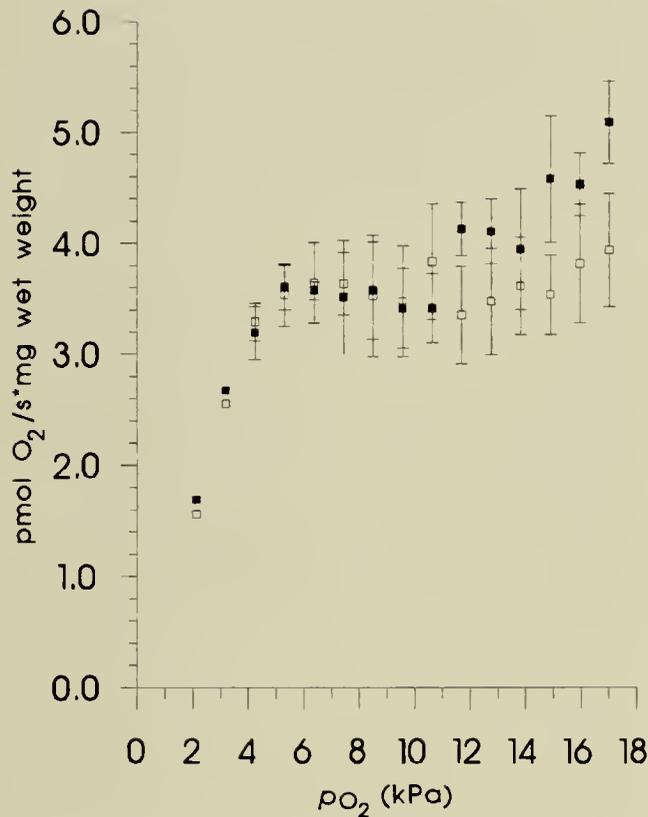


Figure 3. Oxygen consumption rates of juvenile Australian crayfish at various environmental  $p_{O_2}$  when exposed to 0 mg/L (open squares) and 1000 mg/L  $NO_3-N$  (closed squares). Each data point represents the mean  $\pm$  SEM for 5 crayfish.

Indeed, Sanders et al. (1992) demonstrated that, *in vitro*, ammonia can (1) have either no effect on crustacean hemocyanin oxygen binding affinity, or (2) cause a slight increase or decrease in hemocyanin oxygen binding affinity. Either effect depends on pH and the molecular form of ammonia present. In this study, acute  $NH_3-N$  exposure, at 2.5 times the concentration which cause significant mortalities in 12 hr, did not affect the level of oxygen consumption rates or the ability of crayfish to regulate oxygen consumption rates over a wide range of environmental  $p_{O_2}$ . Although not statistically significant, slightly higher oxygen consumption rates were observed when the crayfish were initially exposed to ammonia. These higher rates may have occurred as a result of the chemical detection of ammonia by the crayfish resulting in an excitation response. Oxygen consumption rates did, however, return to rates similar to those of control individuals, suggesting that the mechanism of toxicity of ammonia to juvenile *C. quadricarinatus* may not be directly associated with changes in metabolic rates. Alternatively, a change in oxygen consumption

rates in juvenile crayfish may not have occurred because of the short duration (<1 hr) of exposure to ammonia in this study.

Nitrite substantially reduced the oxygen consumption rates of juvenile Australian crayfish when acutely exposed to concentrations that cause significant mortalities in 4 hr. In fish, nitrite binds to hemoglobin, forming methemoglobin. Methemoglobin has a reduced oxygen binding affinity when compared to hemoglobin, thus causing problems associated with oxygen transport and delivery to tissues. Needham (1961) hypothesized that nitrite can bind crustacean hemocyanin and form methemocyanin, also causing similar respiratory problems with oxygen transport and delivery. Recent evidence suggests that nitrite can bind to crustacean hemocyanin *in vitro* forming methemocyanin (Tahon et al. 1988). Although this effect can be reversed and normal oxygen binding properties of hemocyanin can be restored *in vitro*, the *in vivo* effects of nitrite binding to crustacean hemocyanin are not known. We suggest that the decrease in oxygen consumption rates in juvenile crayfish observed in this study may have occurred as a result of the direct effects of nitrite on hemocyanin oxygen binding affinity. Alternatively, the reduction in oxygen consumption rates may have occurred via some unknown indirect effect on metabolism.

Nitrate, at concentrations up to 1000 mg/L total  $NO_3-N$ , did not affect the level of oxygen consumption rates or the ability of the juvenile crayfish, *C. quadricarinatus*, to regulate oxygen consumption rates at different environmental oxygen tensions. Oxygen consumption rates were, however, slightly elevated in crayfish when they were initially exposed to the higher concentrations and, again, may be related to the chemical detection and excitation. Nevertheless, since no mortalities were observed in juvenile crayfish after exposure to high concentrations for 120 hr, the extent of nitrate's toxic effects appears minimal in *C. quadricarinatus*.

In summary, juvenile Australian crayfish, *C. quadricarinatus*, demonstrate similar tolerances to ammonia, nitrite, and nitrate observed in other crustacean species. The ability of juvenile crayfish to regulate oxygen consumption rates does not appear to be affected by ammonia or nitrate during acute exposure. Nitrite, however, inhibits the ability of juvenile crayfish to regulate oxygen consumption rates. At low environmental  $p_{O_2}$ , this effect can substantially impair respiratory functions and affect the potential survival of juvenile crayfish. Further studies are needed to examine the long term effects of sublethal exposure to ammonia and nitrite on the oxygen consumption rates of this juvenile crayfish and to determine the specific mechanism(s) involved in their toxicity to crayfish and other crustacean species.

#### ACKNOWLEDGMENTS

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# POPULATION BIOLOGY OF THE MUD CRAB, *DYSPANOPEUS SAYI*, AN IMPORTANT PREDATOR OF JUVENILE BAY SCALLOPS IN LONG ISLAND (USA) EELGRASS BEDS<sup>1</sup>

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**ABSTRACT** The xanthid mud crab, *Dyspanopeus sayi* (Martin and Abele 1986), is a common predator of juvenile bivalves in shallow estuaries along the Atlantic coast of the United States. This study assesses spatio-temporal patterns in abundance and size structure of *D. sayi* populations in eelgrass (*Zostera marina*) meadows of eastern Long Island, NY, bays, and their implications for the survival of bay scallops, *Argopecten irradians*, in this critical habitat.

A comparison of suction dredge sampling and diver visual sampling for mud crabs showed that the latter method significantly underestimates densities of this cryptic species. *D. sayi* were extremely abundant, throughout much of the year, in all 4 bays studied, attaining densities of up to 225 crabs m<sup>-2</sup> in eelgrass, but were scarce (mean = 0.5 crab m<sup>-2</sup>) in unvegetated substrates. Densities showed high interannual variation (an order of magnitude increase between 1991 and 1993), and considerable seasonal and micro-habitat variation within a given bay, but little variation (maximum = 2.5-fold) among bays. Recruitment of juvenile mud crabs caused a 3- to 4-fold increase in densities at all sites between late August and early October; these recruits dominated population numbers in early fall but were too small (<10 mm in mean carapace width (CW)) to pose an added threat to natural bay scallops, which are typically >15 mm at this time. Male mud crabs were generally more abundant than females, and achieved morphological sexual maturity at ca. 16 mm CW. Mature males exhibited a significantly greater chela height for a given body size than females, and are thus likely to be capable of crushing larger prey than females of comparable size. Mud crabs in the study area survived to a maximum age of 2 years and CW of 26 mm (i.e., well below the maximum size of 30 mm given for this species). The mean size of adult (1st and 2nd year) cohorts never exceeded 20 mm in CW. Our results, combined with prior work on size-specific predation of *D. sayi* on bay scallops, suggest that scallops gain effective size refuge from natural mud crab populations at ca. 20–25 mm shell height. The bay scallops' rapid summer growth rates (ca. 13 mm month<sup>-1</sup>) relative to *D. sayi* (averaging 1.3 mm month<sup>-1</sup>) allow them to achieve complete size refuge from this predator by late September, within <3 months of larval settlement. Greatest risk of predation by mud crabs (suitable overlap in predator-prey sizes) coincides with the period of off-bottom attachment and exploitation of a partial spatial refuge for scallops within the eelgrass canopy. Population data from this study can be incorporated in future models of scallop population dynamics to quantify predation losses of natural and seeded scallops to mud crabs.

**KEY WORDS:** Mud crab, *Dyspanopeus sayi*, population size-structure, abundance, eelgrass, scallops

## INTRODUCTION

Predation by crabs has often been considered one of the most important sources of natural mortality for juvenile bivalves, and therefore a dominant force in controlling the population dynamics of bivalves (Holland et al. 1980, Morgan et al. 1980, Flagg and Malouf 1983, Jensen and Jensen 1985, Tettelbach 1986, Peterson 1990). In particular, the xanthid mud crab, *Dyspanopeus* (= *Neopanope*) *sayi* (Martin and Abele 1986), has been identified as an important predator of both wild and cultured infaunal, juvenile bivalves, especially the northern quahog, *Mercenaria mercenaria*, in estuaries of the Middle Atlantic Bight (MacKenzie 1977, Flagg and Malouf 1983, Day 1987). The significance of mud crabs as predators of commercially important bivalves may result from their high densities (up to 102 crabs m<sup>-2</sup> in Great South Bay, NY for *N. sayi* and *Panopeus herbstii* combined) (Wapora 1979), relatively high food intake for their size (Elner and Lavoie 1983, Gibbons 1984) and specialized chela morphology, well adapted for crushing hard-shelled prey.

*D. sayi* can also be a serious predator of epifaunal juvenile bivalves, particularly the bay scallop, *Argopecten irradians*. Both predator and prey species have overlapping ranges of distribution along the east coast of the United States: *A. irradians*, from Cape Cod, MA to the Gulf of Mexico (Waller 1969), *D. sayi*, from Nova Scotia to the Florida Keys (Williams 1984), and co-occur in

eelgrass (*Zostera marina*) beds within shallow embayments (Tettelbach 1986). Laboratory experiments have shown that mud crabs can consume juvenile bay scallops at extremely high rates (up to 2.5-mm scallops hr<sup>-1</sup> per 20 mm crab) in the absence of refugia (Strieb 1992). Bauer (1994) further demonstrated in laboratory and field experiments that seagrasses only partially mitigate scallop losses to this predator. In contrast to other crab species, including green crabs (*Carcinus maenas*), spider crabs (*Libinia spp.*), and blue crabs (*Callinectes sapidus*), mud crabs can forage for juvenile bay scallops which are attached off-bottom within the eelgrass canopy (Pohle et al. 1991, Bauer 1994).

Despite the recognized significance of *D. sayi* as a predator on young bivalves, surprisingly little is known about the distribution, abundance and population dynamics, i.e. growth rates, recruitment and mortality rates of natural mud crab populations. *D. sayi* are small, cryptic crabs that are known to prefer and occur at higher densities in heterogeneous, coarse substrate (gravel or crushed shell) than in sand or mud, presumably because it provides them with refuge from their own predators (Day and Lawton 1988). The distribution of the larger xanthid crabs, *P. herbstii* and *Eurypanopeus depressus*, in oyster reefs and intertidal salt marshes has been previously documented (Day and Lawton 1988, Meyer 1994). However, the abundance of *D. sayi* in seagrasses, a common subtidal habitat in many shallow estuaries, has only been determined (by trawling) for the lower Chesapeake Bay, an area where blue crabs, *C. sapidus*, are the numerically dominant crabs (Heck and Orth 1980). Habitat characteristics such as sediment type and presence or absence of submerged aquatic vegetation are

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known to be important in mediating predator-prey interactions (reviewed by Seed 1993). While the physical structure of submerged aquatic vegetation (e.g., Heck and Orth 1980, Orth et al. 1984) and sediment type (e.g., Arnold 1984, Sponaugle and Lawton 1990) can act to directly influence a predator's foraging efficiency, habitat characteristics can also indirectly influence predation through their effects on the distribution and abundance of predators.

Due to mechanical constraints, predation rates by crabs on bivalves are highly size-specific, such that the number of prey consumed and the maximum prey size that can be consumed are often positively correlated with crab size (Whetstone and Eversole 1981, Dare et al. 1983, Peterson 1990). Therefore, information on the size composition of *D. sayi* populations, seasonal growth rates and gender-related differences in size, in addition to crab abundance estimates, are required to assess predation risk of scallops to *D. sayi* in nature. Tettelbach (1986) and Strieb (1992 and unpubl. data) estimated that *D. sayi* can consume bay scallops up to a size approximating their carapace width, such that scallops attain complete size refuge from even the largest mud crabs at ca. 28 mm, a size when scallops in nature permanently lose their ability to maintain position in the eelgrass canopy, and relocate to the bottom (García-Esquivel and Bricelj 1993). In Long Island bays, bay scallops typically settle onto eelgrass and other elevated substrates in early July (Eckman 1987), and grow at a mean rate of ca. 13 mm month<sup>-1</sup> during the summer and early fall (García-Esquivel and Bricelj 1993). The densities and sizes of mud crabs encountered by scallops during the period when they attach to eelgrass, and the relative growth rates of predator and prey, are therefore expected to be important determinants of scallop recruitment success in these bays.

To predict predation risk in nature, determination of size-, temperature- and substrate-specific predation rates must be coupled with that of size structure, abundance, and microhabitat distribution of predator and prey populations in the field. Such information can also be used for bay scallop reseeding programs, which have been increasingly implemented in recent years to counteract declines in bay scallop populations (Peterson and Summerson 1992, Tettelbach and Wenzel 1993). The main objectives of the present study were therefore to determine temporal (seasonal and interannual) and spatial patterns in the abundance and size composition of *D. sayi* populations in eastern Long Island bays, primarily within eelgrass meadows used as nursery habitat by juvenile bay scallops. Annual reproductive events and sex-related differences in mud crab abundance and morphometric parameters were also determined over the course of this study. This and related studies (Strieb 1992, Bauer 1994) will provide a database for future use in modelling wild and seeded bay scallop populations in order to predict site-specific losses to mud crabs and other key predators in relation to the timing of settlement or seeding events in local bays.

#### MATERIALS AND METHODS

Mud crab populations were sampled in eelgrass beds in 4 eastern Long Island bays: Lake Montauk (LM, 71°55'N, 41°4'W), Napeague Harbor (NAPH, 72°3'N, 41°1'W), Northwest Harbor (NWH, 72°15'N, 41°2'W), and Hallock Bay (HB, 72°16'N, 41°8'W) (Fig. 1). All 4 bays are known to have historically supported commercially viable bay scallop populations and have extensive *Z. marina* cover. Lake Montauk beds were sampled ear-

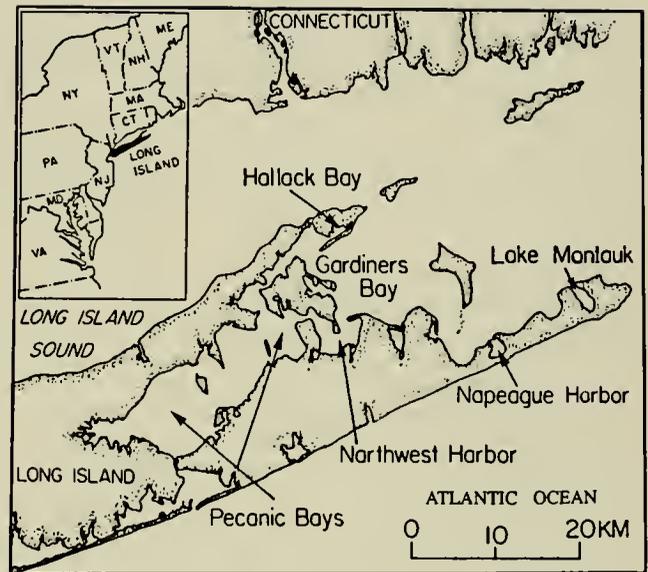


Figure 1. Map showing location of eastern Long Island, New York, embayments where field sampling for mud crabs, *D. sayi*, was conducted.

lier, in June, August and October 1990, whereas the other 3 bays were sampled periodically between July 1991 and June 1992. Additional sampling was conducted in October 1992 and September 1993 in Hallock Bay to determine interannual fluctuations in mud crab densities and population size-structure over a 3-year period. A SCUBA diver-operated suction dredge was used at all locations (with the exception of Lake Montauk) to collect mud crabs in 2-m<sup>2</sup> plots, marked by a PVC frame. Sampling quadrants were deployed haphazardly within the entire vegetated area, and all crabs recovered in the quadrats (typically  $n = 3$ ) were counted in order to estimate mud crab densities for each date and site. The suction dredge was operated by a 5 horse-power motor, had a 9.4 cm diameter intake hose and a 4 mm mesh collection bag secured at the outflow end. Preliminary tests indicated that the suction dredge, which sampled sediments to a depth of ca. 5 cm, was capable of collecting mud crabs of all sizes ( $\geq 1$  mm in carapace width) present, even if they had buried in the sediment.

At Lake Montauk the suction dredge was not used on June and August sampling dates; instead, SCUBA divers swam 1 m-wide, 4 m-long transects ( $n = 9$  to 10) within the eelgrass meadow during daytime, collecting all mud crabs that were found on the bay bottom, in the eelgrass canopy, on elevated structures, burrowed in the sediment, or under shells, pebbles, and vegetation. Crab densities determined by the 2 sampling methods, diver observations ( $n = 5$  transects) and suction dredge samples ( $n = 4$  plots), were compared at this site on October 2, 1990 (analysis of variance [ANOVA] of square root transformed densities, homogeneity of variances tested by the Scheffé-Box test, Sokal and Rohlf 1981). To test for differences between the size frequencies of mud crabs collected by diver vs. suction dredge, a Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1981) was performed on the raw, bimodal frequency distributions.

For each sample we recorded mud crab carapace width (CW, as measured between the fifth anterolateral spines) and for Montauk Lake samples, master chela height (propodus height, the largest chela was always used regardless of handedness). Measurements were taken for all crabs  $\geq 1$  mm in CW. Sex and incidence of

ovigerous females were determined by visual examination of a crab's abdomen (sex could only reliably be determined for crabs >5 mm CW). All mud crabs collected were usually released at the sampling site after measurements were taken.

Size-frequency distributions were constructed for crabs from all collection dates, and modes of CW for each cohort were estimated by the maximum likelihood method of MacDonald and Pitcher (1979). This method assumes a known number of modes, and normality of distributions. The shift of modes over time allowed calculation of growth rates for each mud crab cohort (year class). A paired comparisons test (two-way ANOVA, Sokal and Rohlf 1981) was conducted for each location to compare the mean CW of male and female mud crabs to determine whether there were significant size differences between sexes.

Changes in allometric growth relationships (CW to chela height) have been previously used to determine the size at sexual maturity among various crab species (e.g., Jewett et al. 1985, Conan and Comeau 1986). Furthermore, crab predation rates are sometimes more closely related to chela size than overall body size (Elner 1980). Therefore, morphometric data for the Lake Montauk collections (e.g., regressions of the covariate CW to chela height for male crabs) were analyzed by analysis of covariance (ANCOVA) to identify differences in this relationship between sampling dates. Since significant differences in the slopes of these regressions were not found, all dates were pooled and separate regressions of CW vs. chela height were performed for male and female mud crabs. Sexual dimorphism in body size to chela height was tested by comparing regression coefficients according to methods described by Zar (1984).

#### Crab Densities in Relation to Habitat (Substrate) Characteristics

To determine whether *D. sayi* was restricted to eelgrass habitat, mud crab densities in Napeague Bay were compared between bare (sand) and adjacent vegetated substrate found along the eastern perimeter of this bay between August 1991 and June 1992. Eelgrass beds sampled in Lake Montauk, and Napeague and Northwest Harbors occurred in areas of sandy sediments at depths ranging from 1–3 m. Summer eelgrass densities averaged 575 shoots  $m^{-2}$  at LM (Pohle et al. 1991) and 704, and 464 shoots  $m^{-2}$  at NPAH and NWH respectively (García-Esquivel & Bricelj 1993); canopy heights averaged 41, 38 and 24 cm in LM, NAPH and NWH, respectively.

In Hallock Bay, eelgrass beds are characterized by 2 distinct habitats, hereafter referred to as sandy and muddy habitats. The former lies closer to the inlet connecting Hallock Bay to Gardiners Bay (Fig. 1), in shallower water (0.56 m at mean low water (MLLW), tidal range 0.75 m), and has a relatively shorter, denser canopy. The latter is found in deeper (1.63 m at MLLW), more protected inner bay waters, where the eelgrass canopy can reach up to 1 m in height (Table 1). Detailed characterization of these habitats (contour plots of substrate and eelgrass characteristics) was provided by Strieb (1992). While all mud crabs from Hallock Bay included in the previously described size-frequency distributions were collected within sandy habitat, additional collections were made by suction dredge on the same 5 dates in the muddy habitat. Sampling quadrats were placed haphazardly within each habitat (total vegetated area sampled in Hallock Bay = ca. 500  $m^2$ ). These samples were used to determine differences in crab densities between eelgrass habitats and to investigate mud crab habitat preferences.

TABLE 1.

Characterization of the 2 eelgrass, *Zostera marina*, habitats in Hallock Bay in mid-summer (July/August) 1990.

Eelgrass Habitat	Canopy Height (cm)	Shoot Density (no. $m^{-2}$ )	% Silt-Clay
Muddy eelgrass	62.2 (1.6; 25)	185.0 (12.1; 25)	28.6 (12.3; 11)
Sandy eelgrass	41.5 (2.5; 14)	268.7 (20.6; 14)	10.4 (8.2; 16)

Mean (standard error; n = number of sampled plots) of eelgrass canopy height, shoot density and percent of silt-clay of the sediment.

Eelgrass shoot density was determined within haphazardly selected 2- $m^2$  plots in each habitat, by counting all shoots in at least three 0.068- $m^2$  quadrats per plot (Table 1). Canopy height was determined by measuring the height of the tallest blade for each shoot, for at least 10 haphazardly chosen shoots per plot. Sediments were characterized by taking a minimum of 3 cores from each plot with 50 mL plastic syringes, which were frozen until analysis. Approximately 10 g of surface (top 2 cm) sediment were removed from each core to perform grain size analysis using methods described by Folk (1968).

## RESULTS

The effectiveness of visual censusing by divers versus suction dredge sampling for mud crabs was compared on October 2, 1990 at Lake Montauk (Fig. 2). The densities of mud crabs collected by suction dredge were significantly greater (about 4.5 times higher) than those obtained by diver visual surveys (single classification ANOVA,  $p < 0.05$ ,  $df = 1, 7$ ,  $F = 7.87$ ), but there were no significant differences in the size-frequency distributions of mud crabs obtained with the 2 methods (Kolmogorov-Smirnov two-sample test,  $p > 0.05$ ,  $n_1 = 108$ ,  $n_2 = 290$ ,  $D = 0.132$ ). This indicates that while diver surveys significantly underestimated mud crab densities at this site, they did obtain a representative sample of the population. Based on these results collections at all other sites were made by suction dredge to obtain more accurate estimates of mud crab population abundance.

#### Mud Crab Abundance

Mud crabs were ubiquitous and occurred at high densities throughout the year in eelgrass beds sampled by suction dredge in Northwest Harbor, Napeague Harbor, and Hallock Bay (Fig. 3). Mean densities (all sizes combined) ranged from 7 crabs  $m^{-2}$  on August 12, 1991 to 191 crabs  $m^{-2}$  on September 18, 1993 in Hallock Bay; the highest density observed during the course of this study was 225 crabs  $m^{-2}$  on September 18, 1993 at this site (Figs. 3 and 4). In general, mud crab densities showed relatively low variability (1.2 to 2.5-fold maximum differences) and did not differ significantly among the 3 bays (two-way ANOVA of square root transformed densities with bays and dates as response variables,  $p > 0.1$ ) (Fig. 3), while they differed significantly with sampling date ( $p < 0.001$ ,  $F = 9.36$ ). The same results were obtained when ANOVA was performed on untransformed data. There was also marked inter-annual variability in crab densities in Hallock Bay, where mud crabs showed a general increase in population abundance between 1991 and 1993 (Fig. 4). Densities of adult mud crabs (1st and 2nd year cohorts combined), which due to their large size are expected to pose the greatest threat to juve-

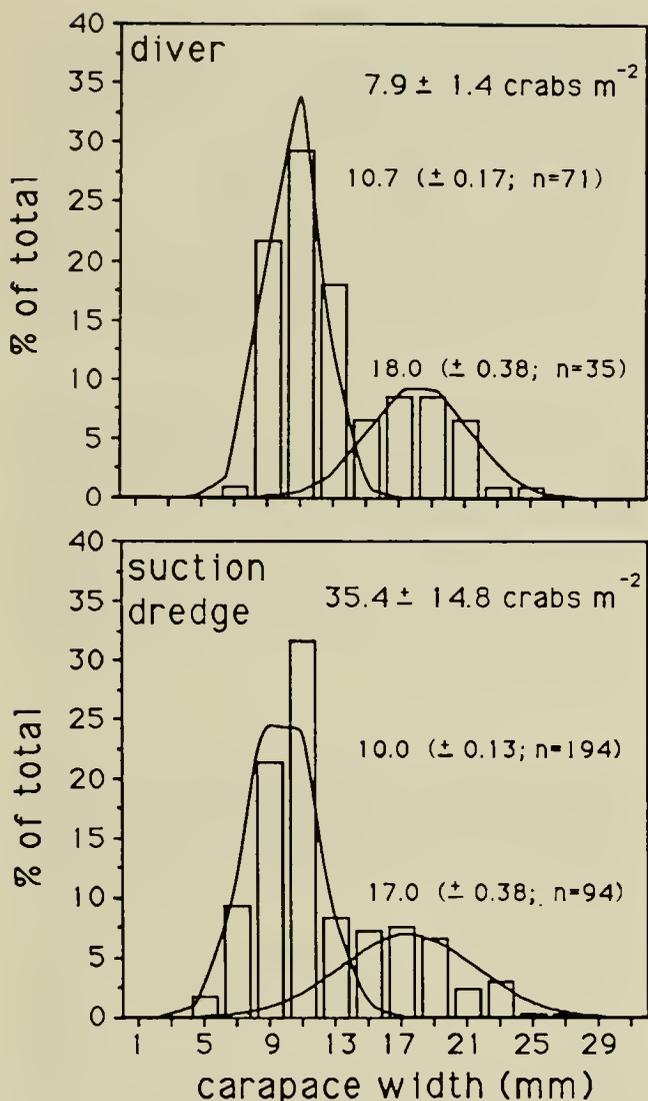


Figure 2. Comparison of size-frequency distributions of mud crab, *D. sayi*, populations collected by diver visual census and a suction dredge in Lake Montauk on October 2, 1990, with fitted normal curves superimposed. Mean carapace width (CW) ( $\pm$ SE) and number of crabs for each cohort are shown for each normal distribution; mean crab densities [ $\text{crabs m}^{-2}$  ( $\pm$ SE)] are also given.

nile bay scallops, were 7, 63 and 36  $\text{crabs m}^{-2}$ , and those of juveniles (cohort 0) were 11, 27 and 154  $\text{crabs m}^{-2}$  in 1991, 1992 and 1993, respectively.

Mud crab densities increased markedly between mid-summer (July to August) and mid-October 1991 at all sites. Analysis of size-frequency distributions (Fig. 3) clearly shows that this 3- to 4-fold increase is associated with recruitment events in all 3 bays sampled. New recruits (mean CW = 3.1 to 4.0 mm) were observed earlier (in late August) at Northwest and Napeague Harbors (the 2 bays found on the south fork of Long Island), than in Hallock Bay. By mid-October, they made up as much as 66 to 76% of the total population at these sites. Thus, only one annual pulse of recruitment, occurring in late summer, was detected with our sampling schedule. Mud crab densities increased again between early April and late June 1992 in Hallock Bay and Napeague Harbor. Ovigerous females were only observed during June, July,

and early August; their contribution to the total female population never exceeded 57% and was greatest in early June (Table 2). The absence of egg-bearing females on the April 1992 sampling dates suggests that the increase in density of mud crabs observed between April and June cannot be attributed to an intervening recruitment event missed by our sampling, and may reflect crab immigration to this eelgrass habitat or spatial patchiness. No information is available, however, on the range of movement of *D. sayi*, although our diver observations suggest that mud crabs do not forage more than a few meters from their burrows during a single feeding excursion.

All parameters used to characterize the eelgrass meadow in Hallock Bay (canopy height, shoot density and % silt-clay content of surface sediment) (Table 1) were found to be significantly different between the mud and sand habitats (single classification ANOVAs,  $p < 0.001$ ). Gravel content (size fraction  $> 2$  mm) averaged 11.3% and 0.6% in the sand and mud habitats, respectively. Mud crab densities differed significantly between habitats ( $p < 0.01$ , two-way ANOVA of square root transformed densities with substrate type and date as factors,  $F[1,20] = 11.23$ ), and were generally 2 to 8 times higher in the sand vs. mud eelgrass habitat in Hallock Bay (Table 3). In Napeague Harbor, where suction dredge samples were also taken in bare sand, mud crabs were absent or rare in unvegetated habitat: zero crabs per  $2\text{-m}^2$  sampling quadrats on August and October 1991 and April 92 sampling dates, and 2  $\text{crabs m}^{-2}$  on the June 1992 sampling.

#### Growth and Population Size Structure

Natural populations of *D. sayi* typically showed unimodal and bimodal size-frequency distributions, representing 0 and 1st year cohorts (Fig. 3). A late August trimodal distribution, reflecting the survival of a second year class, which was presumably the product of a 1989 recruitment event, was only observed in Napeague Harbor. At other sites these older/larger crabs, which averaged only 20 mm in CW, did not survive beyond July. Crabs  $> 22$  mm CW were rare at all locations during the 1991–1992 sampling period (Figs. 2, 3 and 4), and the maximum CW recorded was 26.3 mm in Hallock Bay in 1993. Therefore, in this region, mud crabs die before they reach their asymptotic size of 30 mm (Williams 1984). Gradual merging of juvenile and adult modes resulted in differentiation of 2 adult peaks in Napeague Harbor in April 1992 (Fig. 3).

Seasonal growth rates were estimated from the shift of modes over time only where the identity of a cohort could be reliably ascertained and where recruitment was not acting as a confounding factor (Table 4). Growth rates averaged  $0.9 \text{ mm month}^{-1}$  in the 3 bays sampled in 1991, attaining a maximum of  $1.6 \text{ mm month}^{-1}$ . They were considerably higher, however, up to  $2\text{--}3 \text{ mm month}^{-1}$ , at Lake Montauk the previous year. Apparent decreases in the mean size of a cohort and decreases in mud crab density, observed between October 1991 and April 1992 in the 3 embayments (Fig. 3) may be caused by age/size differential growth and mortality (e.g., higher overwintering mortality of the largest crabs and/or enrichment of the 1st year cohort by some faster growing new recruits). Because of overlap between cohort distributions, it is also possible that incorrect assumptions were made regarding the normality of these distributions, and this would affect the maximum likelihood estimates.

While the timing of recruitment, evidenced by the mean size of new recruits in Hallock Bay over 3 consecutive years (Fig. 4), remained relatively constant from year to year, population size

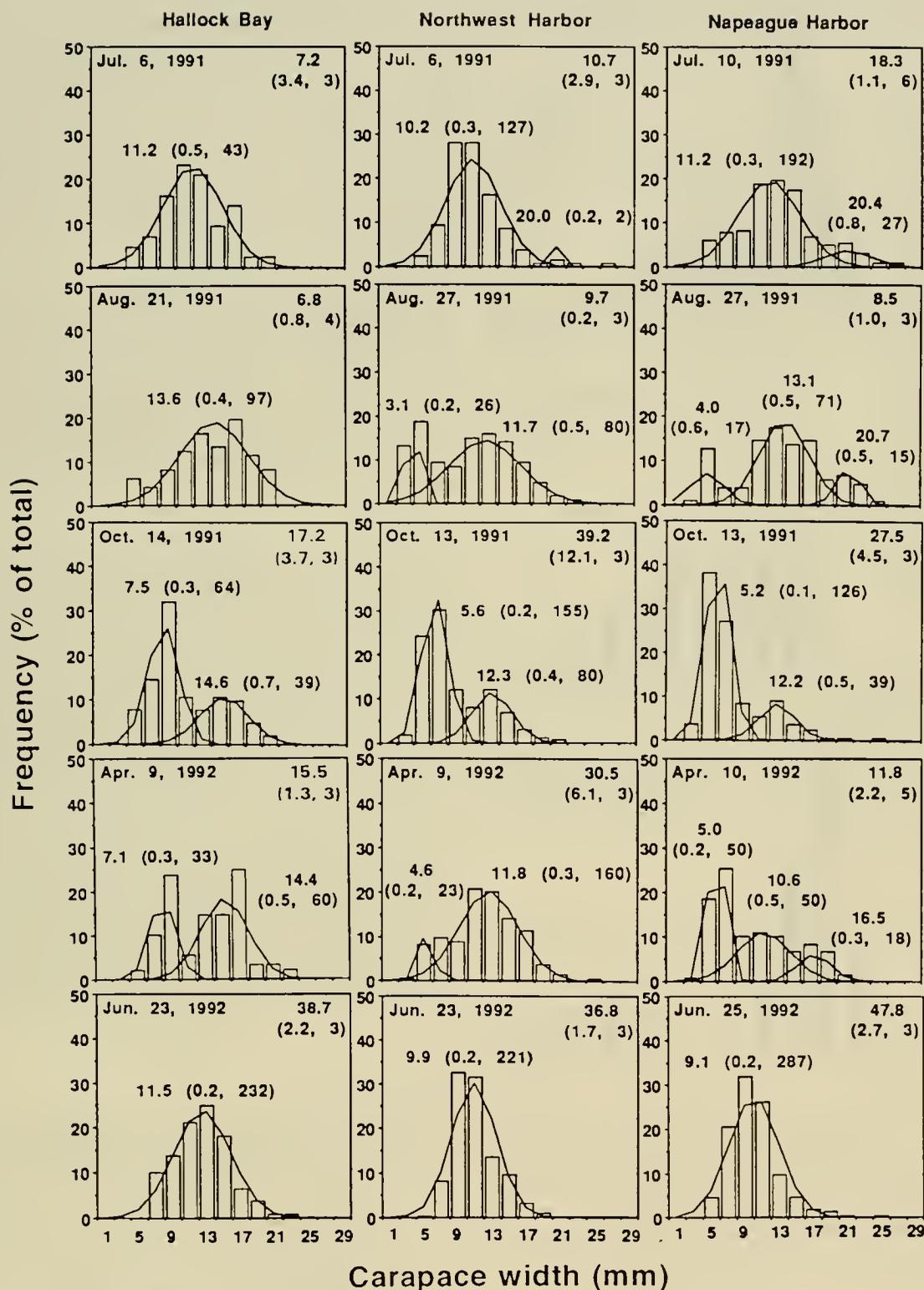


Figure 3. Size-frequency distributions of populations of the mud crab, *D. sayi*, collected by suction dredge during 1990, 1991, and 1992 in eelgrass meadows within three eastern Long Island, NY, embayments, with fitted normal curves superimposed. Mean carapace width in mm (SE, n) is shown for each fitted distribution. Mud crab densities (crabs  $m^{-2}$  [SE, n]) are given in the upper right-hand corner for each sampling date. Surface water temperatures, which differed by  $<2^{\circ}C$  between bays, are given for Napeague Harbor: 23.1, 26.5 and 15.8 $^{\circ}C$  on July, August and October 1991 sampling dates, respectively, and 10.6 and 18.0 $^{\circ}C$  on April and June 1992 sampling dates, respectively.

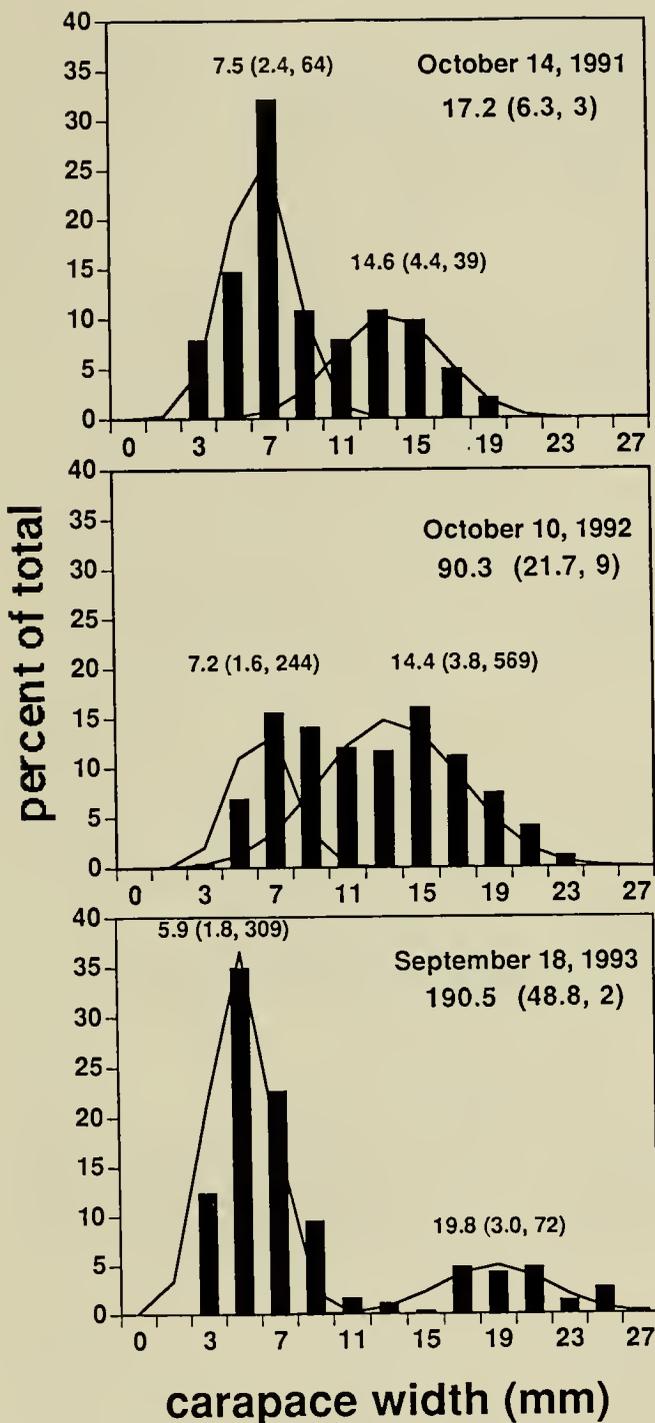


Figure 4. Size-frequency distributions of populations of the mud crab, *D. sayi*, collected during fall 1991, 1992, and 1993 in eelgrass meadows within Hallock Bay, Long Island, NY, with fitted normal curves superimposed. Mean carapace width in mm (SD, n) is shown for each fitted distribution. Mud crab densities (crabs  $m^{-2}$  [SD, n]) are given in the upper right-hand corner for each sampling date.

structure varied considerably between years. Thus the adult (1st year) cohort was significantly larger (mean CW = 19.8 mm) in 1993 than in the 2 previous years (14.4–14.6 mm) ( $p < 0.01$ , a posteriori comparison of means), although it comprised a smaller

percentage of the total population (19% in 1993 vs. 38 and 70% in 1991 and 1992, respectively).

Paired comparisons tests (two-way ANOVAs) conducted in each embayment indicated that there were no significant differences in mean body size (CW) between male and female mud crabs in Northwest Harbor ( $p > 0.75$ ,  $df = 1, 4$ ,  $F = 0.58$ ), Napeague Harbor ( $p > 0.25$ ,  $df = 1, 4$ ,  $F = 1.41$ ), or Lake Montauk ( $p > 0.10$ ,  $df = 1, 3$ ,  $F = 5.45$ ) (Table 2). However, significant sex-related differences in CW were observed in Hallock Bay ( $p < 0.01$ ,  $df = 1, 4$ ,  $F = 27.38$ ), where males were consistently larger, although by only 1 to 2 mm, than females. Males almost completely dominated the larger size classes ( $>20$  mm CW) in Lake Montauk (Fig. 5).

With the exception of one sampling date, sex ratios of male to female mud crabs based on pooled cohorts were always greater than 1:1 (Table 2). The greatest difference between numbers of males and females was observed on August 21, 1991 in Hallock Bay, where there were 2.6 males for each female. Females may experience higher mortalities than males; however, these may not be directly related to brooding since greatest departures from a 1:1 sex ratio (excess males) did not coincide with periods of highest prevalence of ovigerous females.

#### Morphometrics

Regressions of male mud crab CW to chela height in Hallock Bay were not significantly different between dates (ANCOVA performed only for males due to their larger sample size; difference among slopes,  $p > 0.75$ ,  $df = 2, 188$ ,  $F = 0.65$ ; difference among adjusted means,  $p > 0.75$ ,  $df = 2, 190$ ,  $F = 0.17$ ). Therefore, male mud crabs from all sampling dates were pooled to test for morphometric differences between sexes (Fig. 5). Piecewise regression (Systat, Inc., Evanston, Illinois) revealed an inflection point in the relationship between CW and chela height at a CW of 16.0 mm, the morphological maturation size. Since the smallest ovigerous female collected in this study was 6.5 mm CW, these data suggest that males may mature at a larger size (about 16 mm in CW) than females. Significant differences between regression slopes of CW versus chela height were observed for subpopulations of small ( $<16$  mm) and large ( $>16$  mm) male mud crabs ( $p < 0.001$ ,  $df = 294$ ,  $|t| = 36.65$ ), small male and female mud crabs ( $p < 0.001$ ,  $df = 406$ ,  $|t| = 12.8$ ), and large male and all female mud crabs ( $p < 0.01$ ,  $df = 284$ ,  $|t| = 5.33$ ). Thus, overall, male mud crabs had a larger chela than females of a given size.

#### DISCUSSION

##### Mud Crab Abundance

This study shows that *D. sayi* occurs in high densities in eelgrass meadows of four eastern Long Island, NY embayments, although the species exhibited large interannual variations in population abundance at one of the study sites. Mud crab densities were comparable to those obtained by suction dredge in other shallow, Middle Atlantic Bight estuaries such as Great South Bay, NY (maxima of 62 to 102 crabs  $m^{-2}$  for *D. sayi* and *P. herbstii* combined, Wapora 1979) and Lower Norwalk Harbor, CT (8 to 54 crabs  $m^{-2}$ , MacKenzie 1977). These prior studies, however, provide no information on the contribution of different age/size classes to total abundance. Mud crab predation on bay scallops is known to be strongly dependent on predator and prey size (Tettelbach 1986, Strieb 1992). Therefore, mud crab abundance data, in the absence of information on their size composition, are of

TABLE 2.

Sex ratios, percent of all female mud crabs, *Dyspanopeus sayi*, that were ovigerous, and mean carapace width of identifiable males and females (all year classes included) in samples collected in eelgrass beds in four eastern Long Island, NY, embayments during 1990, 1991, and 1992.

Site	Date	Sex Ratio (M:F)	% of Females that Were Ovigerous	Mean CW (SE; n)	
				Females	Males
Lake Montauk	<sup>(a)</sup> Jun. 13, 1990	2.2:1	51.2	10.8 (0.52;41)	12.5 (0.59;92)
	<sup>(a)</sup> Aug. 9, 1990	2.2:1	9.1	10.5 (0.54;33)	13.1 (0.63;73)
	<sup>(a)</sup> Oct. 2, 1990	1.2:1	0	12.3 (0.45;49)	13.9 (0.58;57)
	Oct. 2, 1990	0.9:1	0	12.4 (0.30;151)	12.1 (0.40;139)
Napeague Harbor	Jul. 10, 1991	1.1:1	34.3	11.3 (0.28;102)	14.0 (0.49;108)
	Aug. 27, 1991	1.9:1	0	11.9 (0.46;31)	15.1 (0.59;59)
	Oct. 13, 1991	2.2:1	0	9.4 (0.46;37)	7.7 (0.45;82)
	Apr. 10, 1992	1.5:1	0	9.6 (0.60;43)	9.7 (0.61;64)
	June. 25, 1992	1.2:1	3.8	8.6 (0.18;132)	9.5 (0.27;159)
Northwest Harbor	Jul. 6, 1991	1.5:1	35.3	10.2 (0.41;51)	10.4 (0.42;78)
	Aug. 27, 1991	1.6:1	0	10.4 (0.48;34)	12.7 (0.61;54)
	Oct. 13, 1991	1.9:1	0	9.5 (0.39;67)	8.6 (0.35;125)
	Apr. 9, 1992	1.4:1	0	11.1 (0.35;72)	11.4 (0.41;101)
	Jun. 23, 1992	1.5:1	22.7	9.9 (0.23;88)	10.0 (0.24;133)
Hallock Bay	Jul. 6, 1991	1.4:1	56.5	11.4 (0.44;46)	13.1 (0.52;64)
	Aug. 21, 1991	2.6:1	0	12.4 (0.55;28)	14.4 (0.48;73)
	Oct. 14, 1991	1.3:1	0	10.4 (0.51;62)	11.0 (0.48;80)
	Apr. 9, 1992	1.1:1	0	11.0 (0.50;61)	12.1 (0.55;64)
	Jun. 23, 1992	1.3:1	38.9	11.0 (0.21;113)	12.3 (0.31;149)

All crabs were collected by suction dredge, with the exception of those marked by <sup>(a)</sup>, which were recovered by divers.

limited value in assessing predatory risk for juvenile scallops. For example, the late summer-early fall increase in mud crab abundance, largely attributed to the influx of new recruits < ca. 10 mm in modal CW, does not pose an added threat to natural juvenile scallop populations, which typically exceed 15 mm at this time of year.

The timing of recruitment obtained in this study agrees with that of van Montfrans et al. (1990) for *Neopanope* (= *Dyspanopeus*) *sayi* populations in artificial collectors in Chesapeake Bay, where recruitment occurred between August and October. In their 3-year study (1985 to 1988), maximum postlarval settlement was recorded during September. In addition, Swartz (1975) showed that young-of-the-year mud crabs appeared in July and August in the York River Estuary, Virginia. It is not clear why recruitment

intensity, as well as the density of adult crabs, increased markedly between 1991 and 1993 in the present study (Fig. 4). Eastern Long Island bays have experienced harmful microalgal blooms ("brown tides") with variable frequency, intensity and spatial distribution since 1985. These blooms, which severely reduced bay scallop populations, achieved relatively high concentrations at our study sites during mid-summer 1991, but not in 1990 or 1992-1993

TABLE 4.

Seasonal growth rates (mm shell height month<sup>-1</sup>) of different age/size cohorts of mud crabs, *Dyspanopeus sayi*, in 4 eastern Long Island, New York, embayments.

Site	Time Interval	Cohort (yr.)	Growth Rate (mm mo. <sup>-1</sup> *)
Hallock Bay	Jul. 6- Aug. 21, 1991	1+	1.56
	Aug. 21- Oct. 14, 1991	1+	0.57
Northwest Harbor	Jul. 6- Aug. 27, 1991	1+	0.87
	Aug. 27- Oct. 13, 1991	0+	1.59
	Aug. 27- Oct. 13, 1991	1+	0.39
Napeague Harbor	Jul. 10- Aug. 27, 1991	1+	1.20
	Jul. 10- Aug. 27, 1991	2+	0.18
	Aug. 27- Oct. 13, 1991	0+	0.78
Lake Montauk	Jun. 13- Aug. 9, 1990	1+	2.94
	Aug. 9- Oct. 2, 1990	0+	2.07
	Aug. 9- Oct. 2, 1990	1+	1.95

\* Based on a 30-day month.

Dates for which growth rates could not be determined are not shown.

TABLE 3.

Mean (SD, n) densities of mud crabs, *Dyspanopeus sayi*, in the sandy and muddy eelgrass habitats of Hallock Bay, Long Island, NY, as determined by suction dredge sampling on 5 dates in 1991 and 1992.

Date	Sand			Mud		
	Mean Density (crabs m <sup>-2</sup> )	SD	n	Mean Density (crabs m <sup>-2</sup> )	SD	n
Jul. 6, 1991	7.17	5.84	3	7.00	1.80	3
Aug. 21, 1991	6.75	1.66	4	1.50	0.50	3
Oct. 14, 1991	17.17	6.33	3	7.50	2.60	3
Apr. 9, 1992	15.50	2.18	3	5.33	2.03	3
Jun. 23, 1992	38.67	3.74	3	5.00	1.80	3

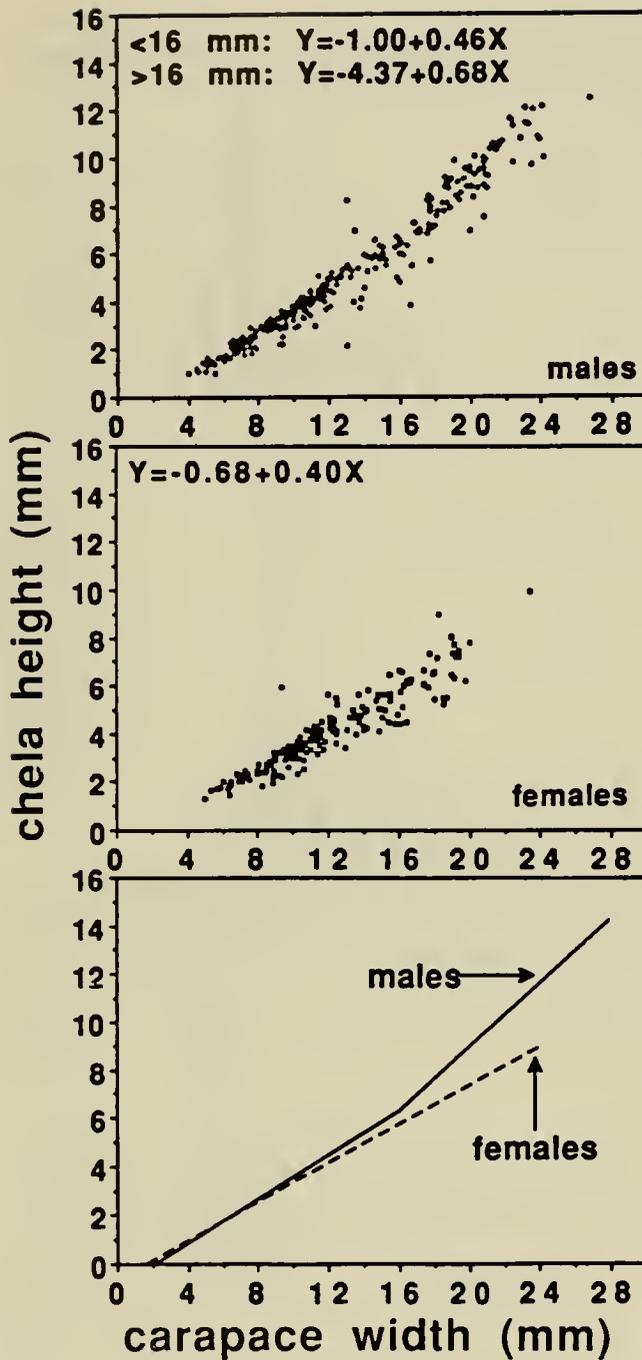


Figure 5. Allometric relationships between carapace width (X) and chela height (Y) of mud crabs, *D. sayi*, (males and females) obtained from pooled samples collected on June 13, August 9, and October 2, 1990 in Lake Montauk, NY. Fitted regression lines follow: Males (<15.98 mm):  $Y = -1.00$  (SE = 0.05) +  $0.46$  (SE = 0.01) X; ( $r^2 = 0.86$ , n = 210). Males (>15.98 mm):  $Y = -4.37$  (SE = 0.19) +  $0.68$  (SE = 0.04) X; ( $r^2 = 0.80$ ; n = 88). Females:  $Y = -0.68$  (SE = 0.14) +  $0.40$  (SE = 0.01) X; ( $r^2 = 0.87$ ; n = 200).

(data from Suffolk County Department of Health, Riverhead, NY). Indirect effects of these blooms (e.g., reduced food availability, and known reduction in eelgrass habitat) (Cosper et al. 1987) on populations of mud crabs and other secondary consumers remain speculative and may merit future consideration.

Mud crabs sampled in Napeague Harbor were found in much higher densities in vegetated compared to unvegetated habitat, supporting earlier findings that these small crabs are cryptic predators that prefer, and are more abundant in, heterogeneous substrates such as gravel (Day and Lawton 1988) and aggregated polychaete tubes (Heck and Hambrook 1991). A higher abundance of *Neopanope* (= *Dyspanopeus*) *sayi* in vegetated (mixed stands of *Z. marina* and widgeon grass, *Ruppia marina*) and unvegetated bottom was also previously noted in the lower Chesapeake Bay (Heck and Orth 1980). However, a close association between *D. sayi* and seagrasses had not been previously documented in Long Island embayments.

Mud crabs were also significantly more abundant in sandy than in muddy eelgrass habitats in Hallock Bay (Table 3). This may be due to higher eelgrass shoot densities in the sandy habitat, which may provide increased shelter from predators or increased availability of epifaunal prey. Alternatively, this finding may be attributed to the higher gravel content observed in the sandy substrate. Flagg and Malouf (1983) found that mud crab abundance in seed clam growout trays correlated positively with increased gravel size, and laboratory experiments indicate that mud crabs prefer gravel and shell substrates to bare sand or mud (Day and Lawton 1988). Crabs may also avoid the muddy habitat because fine-grained particles can clog their gills and reduce respiratory efficiency, although this possibility remains speculative. The observed differences in crab densities may also reflect differential settlement or post-settlement survival between the 2 microhabitats. Overall, mud crab densities showed significant microgeographic variation within a given bay in relation to substrate type, but limited variation among bays, within eelgrass meadows of similar substrate type. Among the eelgrass meadows sampled in this study, however, only that occurring within Hallock Bay showed significant variation in sediment type.

#### Crab Population Size Structure and Implications for the Survival of Bay Scallops

Information on the size structure and recruitment of *D. sayi* populations in eastern Long Island bays (Figs. 3 and 4) suggest that the maximum lifespan of mud crabs in the region is about 2 years. The mean size of adult mud crabs never exceeded 21 mm in CW, and larger crabs were relatively scarce in all bays studied. Food limitation at larger sizes, or predation due to shelter limitation, may contribute to the disappearance of older/larger crabs, since our own observations of wild-caught crabs held in the laboratory indicate that they often attained sizes of up to 30 mm CW.

As indicated earlier, the size composition of mud crab populations described in this study has important implications for the survival of natural *A. irradians* populations. Strieb (1992) showed that consumption rates of *D. sayi* on 20 mm bay scallops in single-prey size laboratory experiments were markedly reduced for all crab sizes tested (11 to 30 mm CW) even in the absence of refuge, compared to those on smaller prey. Therefore, bay scallop populations in eastern Long Island bays are expected to gain a significant size refuge from *D. sayi* at about 20 mm in shell height (SH), well below the absolute size refuge (28 mm) determined in his study. This finding is substantiated by experimental field data. Strieb (1992) found that losses of 20 to 22 mm bay scallops tethered to the base of eelgrass shoots in Hallock Bay (4.5 to 11.4% loss day<sup>-1</sup>) were significantly lower than those of 12 to 15 mm scallops (27 to 40% loss day<sup>-1</sup>) at a time of the year when mud

crabs were identified as numerically dominant predators in the system.

Bay scallops achieve partial refuge from benthic predators by attaching off-bottom within seagrasses (Pohle et al. 1991, Ambrose and Irlandi 1992). At sizes between 15 and 25 mm they gradually relocate to the bottom and lose this spatial refuge; García-Esquivel and Bricelj (1993) found that ca. 65 and 100% of the scallop population in these bays dropped to the bottom at mean sizes of 20 and 28 mm, respectively. Thus, the loss of vertical refuge within the eelgrass canopy in later summer coincides with a marked decline in predatory risk by natural mud crab populations. However, they remain vulnerable to other crab species, such as *C. maenas*, *C. sapidus*, and *Cancer irroratus*, until they attain about 40 mm in shell height (Tettelbach 1986). Diver surveys of likely scallop predators in Hallock Bay in 1990 (Strieb 1992), and Napeague Bay in 1991 and 1992 (Bauer 1994) suggest that mud crabs were indeed the numerically dominant predator in these bays, although daytime diver observations may underestimate the abundance of larger, more mobile crab species, such as blue crabs, spider crabs and lady crabs (*Ovalipes ocellatus*).

The present study determined that mature male *D. sayi* (>16 mm CW) have a larger chela for a given body size than females, thus providing evidence of sexual dimorphism in chela size in this species. Cheliped size is generally positively correlated with mechanical strength and thus the ability of crabs to crush larger hard-shelled prey (e.g., Lee and Seed 1992). Elner (1980) found that differences in chela size of the shore crab, *C. maenas*, between sexes were the major determinant of gender-related differences in preferred prey size. Detection of an inflection point in the cheliped size to CW regression of males, but not females, as observed for *D. sayi* (present study) and *C. maenas* (Lee and Seed 1992), is indicative of a dual, sexual and foraging function of the larger chela in males. Additionally, since in most cases sex ratios of male to female mud crabs in Long Island bays were greater than 1:1, males may contribute disproportionately to predation on juvenile bay scallops, particularly on larger sizes. Swartz (1975), who collected mud crabs from trays of oyster shells, also showed that males dominated the larger size classes of *D. sayi* populations in the York River Estuary, VA, but in contrast to the present study, he found that females were more abundant than males when all sizes were combined.

In vegetated eastern Long Island bays, bay scallops are expected to be most vulnerable to predation by *D. sayi* between initial settlement, occurring typically in early July (Eckman 1987), and early September, when scallops reach an average size of ca. 20 mm (García-Esquivel and Bricelj 1993). This period of their life history coincides with attachment to eelgrass and exploitation of a partial spatial refuge from this predator (Pohle et al. 1991). Furthermore, summer growth rates of mud crabs determined in this study (averaging 1.3 mm mo<sup>-1</sup>) (Table 4) are an order of magnitude lower than those of juvenile bay scallops, thus providing scallops with the opportunity to rapidly outgrow this predator. Both of these factors may prevent mud crab populations from driving local populations of juvenile scallops to extinction. In contrast, sea stars, *Asterias amurensis*, have caused heavy losses of the Japanese scallop (*Patinopecten yessoensis*) in suspended culture, because they enter spat collector bags as larvae and can grow twice as fast as scallop spat (Ventilla 1982).

Results of the present study can be applied towards optimization of bay scallop stock enhancement programs, which rely primarily on the free release of hatchery-produced seed of variable

size in suitable nursery habitat (Tettelbach and Wenczel 1993). Our results, in conjunction with those of Strieb (1992), suggest that bay scallops achieve effective size refuge from predation by naturally occurring mud crabs at ca. 20 to 25 mm SH depending on the site and time of year, and absolute refuge at ca. 28 mm. Therefore plantings of larger juveniles (20–28 mm) would serve to minimize losses to this predator, and mud crab abundance should be considered in selecting sites for planting of small (<20 mm) scallops. Reseeding practices in Long Island bays have indeed shifted to a larger scallop size at planting (from 15–20 mm in 1986 and 1987, to 26–30 mm since 1988), as well as lower stocking densities, in an effort to reduce losses to crab predation (Tettelbach and Wenczel 1993). Although the predator species responsible for heavy losses of small scallops in earlier years were not identified, these changes may have led to the increase in scallop survival rates reported in their study for plantings conducted in October 1988. Controlled experiments in which several scallop size classes are planted simultaneously (Strieb 1992) are required to test the effects of planting size on subsequent scallop recoveries in the absence of other confounding variables (planting season and site).

Mud crabs were found to be persistent members of the eelgrass community in the study area during spring, summer and fall, when sampling was conducted (Fig. 3). It is unlikely that *D. sayi* move offshore to deeper waters during the winter, as documented for larger, more mobile invertebrates (Heck and Orth 1980), since size-frequency distributions determined in our study showed little change between October 1991 and April 1992. Therefore, bay scallops planted at sizes <20 mm are expected to be preyed upon by mud crabs during most of the year, although at rates dependent on seasonal temperature, and eelgrass characteristics (Bauer 1994). Although predation rates of *D. sayi* decline markedly with decreasing temperature (Gibbons 1984), bay scallop growth rates, and thus their ability to achieve refuge in size, are also compromised at lower temperatures occurring during fall and winter (Bricelj and Krause 1992). Thus, although mud crab predation is expected to decrease during the fall and winter (*D. sayi* ceases feeding at temperatures  $\leq 3$ – $5^{\circ}\text{C}$ ) (Gibbons 1984), spatial segregation between predator and prey is never achieved. In contrast, an absolute spatio-temporal refuge is attained by northern quahogs from blue crabs, *C. sapidus* (Peterson 1990), and by bay scallops from the northern puffer fish, *Spherooides maculatus* (Tanikawa and Bricelj, unpubl. data), as both predators migrate seasonally into deeper waters. Future modelling of this predator-prey system will require detailed consideration of temporal changes in relative abundance and size structure of both mud crab and scallop populations, as illustrated in this study, as well as understanding the influence of prey density, habitat refuge, alternative prey, and seasonal temperature on mud crab predation rates.

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## THE FATE OF DOMOIC ACID IN DUNGENESS CRAB (*CANCER MAGISTER*) AS A FUNCTION OF PROCESSING

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**ABSTRACT** High levels (up to 90  $\mu\text{g/g}$ ) of domoic acid, a naturally occurring neurotoxic amino acid responsible for amnesic shellfish poisoning, were found in the viscera of raw Dungeness crabs during the 1991–1992 season. In studies reported here, Dungeness crabs were fed domoic acid contaminated razor clam meats for 6 or 9 days. Analyses of the raw crabs indicated that they rapidly accumulated domoic acid and that the toxin was confined to the viscera, principally in the hepatopancreas (22  $\mu\text{g/g}$ ). No domoic acid was detected in either body or leg meats of the raw crabs. When the whole crabs were cooked in either fresh or salted water, using a typical commercial cooking process, visceral domoic acid concentrations were reduced by 67–71%. After processing, the toxin was found outside of the visceral cavity in the body (1.9  $\mu\text{g/g}$ ) and leg (1.1  $\mu\text{g/g}$ ) meats. The majority of the domoic acid was extracted out and diluted into the cook water (0.4–0.8  $\mu\text{g/ml}$ ). The effect of storage conditions, typical of those used following processing and distribution, are also reported. Cooked crabs were held 1 day at 1°C and then analyzed; domoic acid was detected in the viscera and body meats. Portions of the body meat proximal to the viscera had higher domoic acid levels than those distal, but the toxin levels never exceeded 2.1  $\mu\text{g/g}$ . This was also the case in crabs held for 6 days at 1°C and can be explained by simple diffusion. No domoic acid was found in leg meats of the 1 and 6 day storage samples. After storage for 90 days at –23°C, domoic acid was then found in very low concentrations outside of the viscera in the body (<0.8  $\mu\text{g/g}$ ) and leg (<0.4  $\mu\text{g/g}$ ) meats of the crabs. Our results indicated that the storage conditions had some affect on the toxin distribution throughout the cooked crabs, but had no affect on the total domoic acid burden in each Dungeness crab.

**KEY WORDS:** Domoic acid, amnesic shellfish poisoning (ASP), Dungeness crab, *Cancer magister*, razor clams, *Siliqua patula*

### INTRODUCTION

Domoic acid (DA) is the naturally occurring neurotoxic amino acid responsible for amnesic shellfish poisoning (ASP) (Quilliam and Wright 1989, Todd 1993). This marine biotoxin causes gastrointestinal and neurological symptoms in humans within 24–48 hours of consumption, and symptoms range from simple nausea and vomiting to headache, confusion, permanent short-term memory loss, coma, and even death. Since 1987, studies in Canada and elsewhere have found that domoic acid can originate from phytoplankton, specifically the diatom genus *Pseudonitzschia*. To date, domoic acid has been found to be produced by at least 4 of the 21 species of *Pseudonitzschia* diatoms: *P. pungens* forma *multiseriis* (Hasle) Hasle, *P. australis* Frenguelli, *P. delicatissima* (Cleve) Heiden, and *P. seriata* (Cleve) Peragello (Lundholm et al. 1994).

The first outbreak of domoic acid poisoning in the United States occurred in September of 1991. While no human illnesses similar to those reported in the 1987 Canadian outbreak occurred, the toxin caused more than 100 pelicans (*Pelecanus occidentalis* Linnaeus) and cormorants (*Phalacrocorax penicillatus* Brandt) to become ill or die. The seabirds had been feeding on anchovies (*Engraulis mordax* Girard), which had grazed on a bloom of *P. australis* (Work et al. 1993). Samples of these anchovies were analyzed in our laboratory and found to contain up to 485  $\mu\text{g DA/g}$  viscera (Wekell et al. 1994a).

Shortly thereafter, domoic acid was discovered throughout the Pacific coastal states of Oregon and Washington in October of 1991. An extensive survey of coastal marine life was performed,

and the toxin was found in anchovies, blue mussels (*Mytilus edulis* L.), razor clams (*Siliqua patula* Dixon), and Dungeness crabs (*Cancer magister* Dana). During the toxin's peak in Washington, our laboratory reported up to 230  $\mu\text{g DA/g}$  meat tissue (average = 106  $\mu\text{g/g}$ ; N = 36) in razor clams, and up to 90  $\mu\text{g DA/g}$  raw viscera (average = 15  $\mu\text{g/g}$ ; N = 87) in Dungeness crabs. The source of domoic acid in Washington and Oregon was never confirmed; however, *P. australis*, *P. pungens* f. *multiseriis*, and *P. pseudodelicatissima* (Hasle) Hasle have been identified in waters of the Pacific Northwest (Homer 1993, pers. comm.). Human illnesses were avoided because of the timely action of local, state, and federal agencies identifying the threat and closing the affected fisheries until they were considered safe (i.e., below 20  $\mu\text{g DA/g}$ ). During 1992, the legal limit for crab viscera was raised to 30  $\mu\text{g DA/g}$  (U.S. FDA 1993). These closures and subsequent restrictions have had significant financial impacts on both the fisheries and the supporting communities.

The Dungeness crab industry is an important component of the economies of the Pacific coastal states. In Washington, Oregon, and California there are over 1,200 crab fisheries. This industry lands an average of \$32 million pounds of crab per season, results in an average annual post-processing value of \$112 million, and contributes significantly to employment in otherwise economically depressed areas (Didier 1995).

It was because knowledge concerning domoic acid production and movement through the marine food web was extremely limited, that state and federal risk managers had little choice but to implement total closures of the Dungeness crab and razor clam

fisheries in these states in December 1991. In order to help re-open these fisheries, our laboratory participated in surveys and lot analyses of Dungeness crab from Oregon and Washington, and samplings of razor clams from the Washington coast. This data provided a model that indicated the breadth and depth of domoic acid contamination in both Dungeness crab and razor clams.

In our analyses, we examined only raw or un-cooked molluscan shellfish and crabs (Wekell et al. 1994a, b). In these investigations, we found that domoic acid was confined to the digestive system in the live or uncooked crabs. We also observed that levels of domoic acid in cooked crabs were considerably lower than in raw crab samples from the same area. It appeared that processing the crabs reduced domoic acid levels. In order to test this hypothesis, we conducted several studies on Dungeness crab contaminated with domoic acid.

### EXPERIMENTAL APPROACH

The use of naturally contaminated Dungeness crabs in these studies was not possible since, at the time (spring and summer of 1994), domoic acid levels in crab had declined to very low levels (i.e., typically 0–5  $\mu\text{g DA/g}$  raw viscera). In addition, the variability from animal to animal was extremely high (Wekell et al. 1994a). This high variance would have required very large numbers of animals to detect statistical differences expected in such a study. Previous work in our laboratory indicated that crabs could be manually fed razor clams naturally contaminated with domoic acid. The resulting variation of toxin levels between animals in the lab was significantly smaller than that found in the natural state. In these studies it was possible to follow on a daily basis the uptake and depuration of domoic acid by the Dungeness crabs. (Lund, in prep.) This presented an opportunity to investigate crabs held under laboratory conditions and fed a portion-controlled diet containing known concentrations of domoic acid.

In a pilot study, we fed Dungeness crabs toxic razor clams and sampled a subset of the crabs daily. The raw crabs were dissected into sections (individual organs of the viscera, body, and leg meats) and all parts were analyzed for domoic acid. From this pilot study, we determined how much of the clam meat the crab would eat per feeding, as well as the rate and distribution of toxin uptake. The results confirmed that in the raw Dungeness crabs, no domoic acid is detected outside of the viscera; and the toxin remains exclusively in the hepatopancreas and the digestive track (data not shown).

After the pilot study, 2 experiments were performed to determine whether domoic acid migrates from the viscera of Dungeness crabs into the cook water and/or into the meat as a result of cooking and post-processing conditions. In the first experiment, the goal was to investigate the fate of domoic acid as a result of cooking the Dungeness crabs in either fresh or salt (3% NaCl) water. In the second experiment, the objective was to investigate the fate of domoic acid in cooked crab as a result of post-processing storage conditions.

### MATERIALS AND METHODS

#### *Reagents*

Methanol (MeOH) and acetonitrile (MeCN) were HPLC-grade (Baxter Healthcare Corp., Burdick and Jackson Division, Muskegon, MI 49442). The trifluoroacetic acid (TFA) was obtained from Sigma (Sigma Chemical Company, St. Louis, MO 63178). The sodium chloride (NaCl) and all other reagents were

analytical grade. All solutions were prepared with distilled de-ionized water (Milli-Q, Millipore, Bedford, MA 01730).

#### *Standards*

Domoic acid was obtained as a certified standard (DACS-1, Canadian National Research Council, Institute of Marine Biosciences, 1411 Oxford Street, Halifax, N.S., Canada B3B 3Z1), and as a 90% pure reagent from Sigma. The DACS-1 was used to prepare a 2.00  $\mu\text{g DA/ml}$  10% aqueous MeCN instrument calibration standard, and the 90% pure reagent was used to prepare a 20  $\mu\text{g DA/ml}$  50% aqueous MeOH quality assurance sample. The standards were stored in the refrigerator when not in use.

#### *Razor Clams*

Clean, non-toxic razor clams were purchased from a local supermarket. The clams were obtained from a supplier in Alaska and were analyzed for domoic acid content in our laboratory. Levels of domoic acid in these clams were non-detectable. The non-toxin bearing clams were fed to the crabs during the first 24–48 hours of captivity to permit acclimatization to the laboratory environment and feeding regime.

The toxin source for the feed in these experiments were raw, frozen, canned razor clams. The clams had been harvested during the 1991 domoic acid incident along the west coast and were embargoed because of the high domoic acid content. We were permitted to purchase the clams from a commercial supplier in Oregon for experimental purposes. The frozen cans were sampled by coring, and analyzed. The toxic clam cores averaged 40.3  $\mu\text{g DA/g}$  tissue (range: 36.3–50.2  $\mu\text{g/g}$ ; N = 4 cans) for Experiment 1; and 57.1  $\mu\text{g DA/g}$  tissue (range: 48.1–62.2  $\mu\text{g/g}$ ; N = 10 cans) for Experiment 2. Daily feed composites samples were also collected during the experiment and analyzed; these samples averaged 27.6  $\mu\text{g DA/g}$  tissue (range: 25.6–31.4  $\mu\text{g/g}$ ; N = 6) for Experiment 1 and 45.5  $\mu\text{g DA/g}$  tissue (range: 34.6–53.8  $\mu\text{g/g}$ ; N = 9) for Experiment 2. The difference in DA concentration of the feed core samples and the daily feed samples was due to the loss of DA in the clam drip or thaw liquid.

#### *Dungeness Crab*

To insure our test animals were free of domoic acid, we acquired live Dungeness crabs caught in the Pacific Ocean off the Canadian and Alaskan coasts in areas where the toxin has not been detected. We purchased 56 crabs for Experiment 1, and 73 for Experiment 2 from a company in Washington state. A subset of 6 to 12 crabs were randomly selected prior to each experiment and individually analyzed for domoic acid to confirm the absence of toxin.

#### *Conditions*

The Dungeness crabs were transported from the purchasing site in chilled coolers and then held in large glass or fiberglass tanks at our Mukilteo, WA facility. The tanks were filled with sand and gravel filtered Puget Sound sea water (12.2–13.3°C) and continuously refreshed with a flow-through system at a rate of 4–6 L/min. Each tank held 8 to 9 crabs.

### EXPERIMENTAL OUTLINE

#### *Crab Feeding*

The crabs were labeled with a number and acclimated to their new environments for 24–48 hours; during this time they were fed

a small amount of clean razor clam meat ( $\leq 12$  g). This initial feeding and time interval allowed for natural fatalities to occur before the experiment began, and permitted identification of obvious and possible poor eaters.

The crabs were then hand fed 10 to 18 g of toxic razor clams using long-armed utility claws for 6 (Experiment 1) and 9 (Experiment 2) days, excluding weekends. Experiments 1 and 2 were carried out over a period of 9 and 13 days, respectively. The goal of these feedings was to obtain crabs with viscera domoic acid contents above 20  $\mu\text{g/g}$ . The amount of clam meat and number of days to feed were determined accordingly. During the experiments each group of crab was fed an average of 30.7 mg DA (Experiment #1) and 55.3 mg DA (Experiment #2).

As each experimental study proceeded, dead or dying crabs, and poor or non-eating crabs were removed from the tanks. When the feeding was complete, the crabs were randomly divided into experimental groups; for each study, all groups contained 12 crabs. The crabs were removed from the tanks, packed in coolers and transported within 1 hour to our Seattle, WA facility. Upon arrival at our laboratory, the crabs were immediately sacrificed and processed (either as raw samples or utilized in the experimental cooking and/or storage studies).

#### Experiment 1: Fresh vs. Salt Water Cooking

The object of this study was to determine if cooking in salt water (3% NaCl) or fresh (non-salted) water had an effect on the resulting domoic acid content in the crab. In the study, the crabs were divided into 3 groups: raw control crabs, fresh water cooked crabs, and salt water cooked crabs. The cooked crabs were boiled for 20 minutes in 18 gallons of either fresh or salt water, using a steam-jacketed stainless steel commercial cooker. After cooking, the crabs were rinsed briefly with cold water, drained, and allowed to cool. Caution was taken to place each crab in the same orientation during the cooking and cooling processes. Once cool, a composite of the drip was collected from each cooked group. All the crabs were then dissected into viscera (hepatopancreas only), body and leg (merus) meats. The samples were refrigerated ( $1^{\circ}\text{C}$ ) less than 24 hours and analyzed.

#### Experiment 2: Post-Processing Storage Conditions

The object of this study was to determine the effects, if any, of storage on domoic acid content of cooked crab. In this experiment, the crabs were divided into 4 groups: raw control crabs, salt water cooked crabs held 1 and 6 days at  $1^{\circ}\text{C}$ , and salt water cooked crabs held 90 days at  $-23^{\circ}\text{C}$ . Three groups of crabs were boiled for 20 minutes in 27 gallons of salt water. Again, the crabs were cooked, rinsed, drained, and allowed to cool with special attention paid to crab orientation. Each crab was then individually bagged and placed vertically (posterior up) on large trays. The groups were stored for 1 day at  $1^{\circ}\text{C}$ , 6 days at  $1^{\circ}\text{C}$ , or 90 days at  $-23^{\circ}\text{C}$  before sampling. The drip was collected, if available, and the crabs were dissected into viscera (all of the gut soft tissues, excluding gills and heart), body meat proximal and distal to the viscera (the meat between the visceral cavity and the leg knuckles was divided into two equal parts), and the leg meat (merus and knuckle meat from the first walking leg). All samples were analyzed immediately after the allotted storage time had expired.

#### Sample Preparation

All tissues collected in the studies were homogenized using a common household blender. To ensure thorough homogenization,

the meat samples (body or leg) were mixed 1:1 (wt:wt) with distilled water prior to blending. Allowances were made for this added water in the final calculations of domoic acid concentration. Visceral tissues were homogenized without the addition of water.

#### Domoic Acid Analysis

All samples were analyzed by the methanol extraction method of Quilliam et al. (1989, 1991) with modifications to the solid phase extraction (SPE) clean-up step (Hatfield et al. 1994). All analyses were performed on a Hewlett-Packard 1090 High Performance Liquid Chromatograph (HPLC) equipped with a Vydac 201TP column (Reversed phase  $\text{C}_{18}$ , 2.1 mm  $\times$  25 cm, Separations Group, Hesperia, CA 92345), and a diode array detector set at 242 nm with a 10 nm bandwidth. The domoic acid was chromatographed isocratically at  $40^{\circ}\text{C}$  with  $\text{H}_2\text{O}/\text{MeCN}/\text{TFA}$  (90/10/0.1) (v/v/v) at a flow rate of 0.300 ml/min. Sample injections of 20  $\mu\text{L}$  were used, and the domoic acid retention times were between 7 and 9 minutes. A 2.00  $\mu\text{g/ml}$  DACS-1 standard was included before, after, and within sets of samples for calibration and quantitation. A 20  $\mu\text{g}$  DA/mL quality assurance sample was always included to assure that full ( $>90\%$ ) recovery of the domoic acid was obtained from the SPE step.

## RESULTS AND DISCUSSION

#### Experiment 1: Fresh vs. Salt Water Cooking

The effect of salt in the cook water during processing of the Dungeness crab was examined. After the 6 feedings with contaminated razor clams, the raw control group indicated the crabs attained an average of 22.1  $\mu\text{g}$  DA/g hepatopancreas. Based on the mass balance analysis of the raw crab viscera, approximately 42.3% of the total domoic acid feed dose was retained by the Dungeness crabs. After processing, the fresh and salt water cooked crabs yielded domoic acid concentrations of 6.39 and 6.43  $\mu\text{g/g}$ , respectively (Table 1). When considering the original DA concentration in  $\mu\text{g/g}$ , there was a 71.1% reduction in domoic acid in the hepatopancreas after cooking the crabs in fresh water, and a 70.9% reduction after cooking the crabs in salt water. Looking at the absolute value of micrograms of DA content in the hepatopancreas, there was an average loss of 78.8% in the fresh water and 79.7% in the salt water cooked crabs. Therefore, the addition of salt had no effect on the resulting domoic acid concentration in the crabs.

Domoic acid is a highly water soluble, low molecular weight amino acid (Falk et al. 1991). Once the physiological and biological barriers in the crab are disrupted and their integrity compromised during the cooking process, the toxin would be expected to migrate throughout the crab and into the cook water. Direct analysis of the cook waters by HPLC indicated 0.81  $\mu\text{g}$  DA/ml for the fresh cook water and 0.72  $\mu\text{g}$  DA/ml for the salt water. This would partially explain the large reduction in the visceral domoic acid content; although we can not eliminate the possibility of chemical alteration of the DA (i.e., loss of the molecule's UV chromophore).

#### Experiment 2: Post-Processing Storage Conditions

The effect of post-processing storage conditions on domoic acid concentration and distribution in the Dungeness crab was examined. Based on the raw control group (Figure 1), the crabs in this study attained an average domoic acid concentration of 22.4

TABLE 1.

Experiment #1: Dungeness crab weight (g) and domoic acid (DA) concentration ( $\mu\text{g/g}$ ).

Group	Statistics	HP Wt. (g)	HP DA conc. ( $\mu\text{g/g}$ )	BR or BC Wt. (g)	BR or BC DA conc. ( $\mu\text{g/g}$ )	LC Composite Wt. (g)	LC Composite DA conc. ( $\mu\text{g/g}$ )
Blank (raw, non-fed)	Ave:	NA	0	NA	0	NA	0
Raw	Ave:	49.41	22.08	NA	0	NA	0
	Std Dev:	7.23	7.89		0		
	%CV:	14.63	35.72				
	Low:	35.90	6.46		0		
	High:	61.94	33.00		0		
Fresh water cooked	Ave:	36.05	6.39	63.09	1.88	126.5	1.10
	Std Dev:	8.16	1.68	11.94	0.59		
	%CV:	22.64	26.34	18.92	31.53		
	Low:	25.7	3.57	49.34	1.03		
	High:	51.77	9.23	83.54	3.12		
Salt water cooked	Ave:	34.37	6.43	66.82	1.86	120.9	1.11
	Std Dev:	6.55	2.00	8.27	0.42		
	%CV:	19.06	31.05	12.38	22.40		
	Low:	22.83	4.54	53.36	1.34		
	High:	46.2	11.24	80.80	2.80		

NA = Not Available; HP = Hepatopancreas; BC = Body meat cooked; BR = Body meat raw; LC = Leg meat cooked (composite of the merus meat of the first walking leg from each of the 12 crab); Ave = average or arithmetic mean; Std Dev = standard deviation about the mean; %CV = percent coefficient of variation (standard deviation divided by the mean times 100).

$\mu\text{g DA/g}$  viscera and retained approximately 47.3% of the total domoic acid feed dose. No domoic acid was found outside of the viscera in the raw crabs. Again, there was a reduction (67%) in viscera domoic acid  $\mu\text{g/g}$  concentrations after cooking (Table 2). Domoic acid burden, total  $\mu\text{g}$  of domoic acid per crab, was reduced by nearly 68%. The toxin lost was detected in the cook water at a concentration of 0.4–0.6  $\mu\text{g DA/ml}$ , and in the drip at 1.25  $\mu\text{g DA/ml}$ . The crab meat sections also had detectable amounts of domoic acid (Figure 1).

The length of storage after cooking had little effect on the absolute domoic acid concentration in the crabs. When all 3 storage conditions were compared, no significant differences ( $p <$

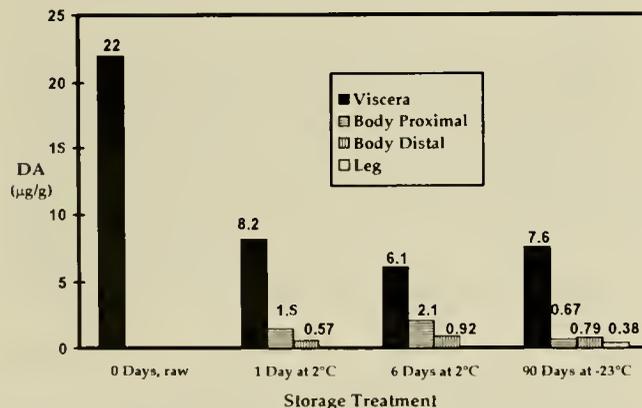


Figure 1. Experiment #2: Domoic acid (DA) distribution and concentration ( $\mu\text{g/g}$ ) in cooked Dungeness crab resulting from different post-processing storage conditions (length of time held after cooking and temperature); referenced to raw Dungeness crab analyzed immediately after sampling.

0.05) were found in the domoic acid concentration in the viscera and the distal body meats. There were, however, differences detected between the toxin levels in the proximal body and leg meats in the 3 groups. Domoic acid was only detected in the leg meats from the samples frozen at  $-23^\circ\text{C}$  for 90 days.

The freezing and thawing of the crabs had an effect on the distribution of the domoic acid within the crab. With the crabs held for 1 or 6 days at  $1^\circ\text{C}$ , there was a relationship between the domoic acid concentration in the meat and its proximity to the viscera. The closer the body meat was to the viscera, the higher the domoic acid concentration. These observations can be explained by assuming a simple diffusion model. This did not seem to be the case with the 90-day frozen samples; the toxin appeared randomly distributed throughout the crab. Regardless of the domoic acid distribution within the crabs, the resulting absolute domoic acid content in the

TABLE 2.

Experiment #2: Average domoic acid (DA) content ( $\mu\text{g}$ ) and concentration ( $\mu\text{g/g}$ ) in raw and cooked Dungeness crab viscera.

Group (N = 12)	Total Viscera DA Content ( $\mu\text{g}$ )	Average Absolute % Loss ( $\mu\text{g}$ )	Average Viscera DA Conc. ( $\mu\text{g/g}$ )	Average Relative % Loss ( $\mu\text{g/g}$ )
RAW CRAB				
No storage	26,194.4		22.38	
COOKED CRAB				
1 day at $1^\circ\text{C}$	9,176.0	65.0	8.18	63.4
6 days at $1^\circ\text{C}$	8,016.7	69.4	6.11	72.7
90 days at $-23^\circ\text{C}$	8,102.4	69.1	7.64	65.9
Ave:	8431.7	67.8	7.31	67.3

TABLE 3.

Experiment #2: Estimated absolute domoic acid (DA) content ( $\mu\text{g}$ ) in cooked Dungeness crab stored under three different treatment conditions.

Cooked Group (N = 12)	Total DA Content ( $\mu\text{g}$ )
1 day at 1°C	10,502
6 days at 1°C	10,122
90 days at -23°C	10,006
Ave:	<b>10,210</b>
Std Dev:	<b>259</b>
% CV:	<b>2.5</b>

cooked Dungeness crabs was essentially the same for all 3 treatment groups (Table 3).

### CONCLUSIONS

These studies indicated that the concentration of domoic acid taken up by the Dungeness crabs during feedings was decreased by 67–71% during the cook processing. The bulk of the domoic acid was extracted from the crabs and diluted into the cook water and drip. After cooking, the major portion of the domoic acid remaining in the crabs was found in the viscera. Only very low levels of the toxin were detected in the body or leg meats. Storage time and temperature did not affect the overall burden of the toxin in the crab; although freezing/thawing did change the distribution of the domoic acid within the crab.

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## THE GONOPOD TEGUMENTAL GLANDS OF SNOW CRAB (*CHIONOECETES OPILIO*) ARE ACCESSORY REPRODUCTIVE GLANDS

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**ABSTRACT** To date, the role of the tegumental glands found in the first gonopod of brachyuran crabs has been a matter of conjecture. In order to more clearly understand the nature and ultimate function of these glands, histological and histochemical studies were performed on 17 male snow crabs, *Chionoecetes opilio* (O. Fabricius), captured in the Baie des Chaleurs, New Brunswick, Canada. Mature (M) and immature (IM) individuals were differentiated based on the carapace width (CW): cheliped height (CH) ratio. To assess the developmental trajectory of the glands, the immature crabs were subdivided into 3 groups, small immature (<40 mm CW), medium immature (40–70 mm CW), and large immature 70–100 mm CW). The precise distribution of the glands within the first gonopod was determined via serial sections (7–10  $\mu$ m). The following histochemical tests were performed on the subsequently revealed glandular region of the first gonopod: Sudan black and Nile blue for lipids, orange G for aminated substances, alcian blue for acid mucopolysaccharides, and periodic acid-Schiff for neutral mucopolysaccharides (MPS). The volume fraction of the gonopod glandular region occupied by glands was assessed using stereologic counts.

The glands were determined to be of the rosette type, and restricted to a specific region at the base of the endopodite. Ducts leading from these glands to the cuticle of the ejaculatory canal only were clearly visible in medium immature to mature individuals; these ducts connected to pores in the cuticle. Cuticular pores and ducts were not observed in small immature crabs. The volume fraction of the glands increased in each successive maturity category, with a mean of 0.8% in small immature crabs and a mean of 8% in mature crabs. The glands contained either acid or neutral mucopolysaccharides, or a mixture of both. The pores of the ejaculatory canal contained similar secretions. These observations support the conclusion that the first gonopod tegumental glands in *C. opilio* are accessory sex glands.

**KEY WORDS:** *Chionoecetes*, reproduction, gonopod, tegumental gland

### INTRODUCTION

Majid crabs of the genus *Chionoecetes* are a major fishery species of the Northern hemisphere (Anonymous 1989); in Atlantic Canada, the snow crab (*Chionoecetes opilio*) fishery accounted for catches of 18,580 MT in 1993, with a commercial value of nearly \$140 million (Department of Fisheries and Oceans 1995). As a relatively recent fishery (begun in the 1970s), it was eventually recognized that a firm understanding of reproductive processes was necessary for the enlightened management of snow crab stocks (Bailey and Elner 1989). Anatomical, histological, behavioral, and physiological studies have progressively elucidated the complex reproductive biology of this species, which is characterized by several potential pathways (for review, see Elner and Beninger, in press).

As in all decapods, fertilization in snow crab is accomplished via the male's first and second pleopods, which are variously modified to form gonopods (Bauer 1986). Despite their obviously crucial role in reproduction, as well as their significance in taxonomic determination and phylogeny (Hartnoll 1975, Chambers et al. 1980, Bauer 1986, Martin and Abele 1986), brachyuran gonopods have been the object of surprisingly little study (see Beninger et al. 1991 for review and references). The functional anatomy and histology of the mature snow crab gonopods were documented by Beninger et al. (1991). Among other findings, the presence of a rosette-type glandular system was reported within the first gonopod. This system was present in the first gonopod only, and was shown to be connected to pores in the ejaculatory

canal cuticle; other cuticular regions were not linked to the glands and contained no pores. These observations led Beninger et al. (1991) to propose that the glandular system was related to reproductive function, and being outside the anatomical continuum of the testes-vas deferens, they could therefore be considered accessory sex glands.

The vocabulary and ascribed functions of glands associated with the crustacean exoskeleton were somewhat confusing (see Beninger 1991 for discussion of this point), until Talbot and Demers (1993) published a comprehensive review of the subject. It was thenceforth clear that the glands of the snow crab first gonopod were tegumental glands of the rosette type. Debate continued, however, concerning their functional status as accessory sex glands, and in a recent review, Subramoniam (1993) concluded that even more data would be required before assigning such a status to these glands. In essence, it would be necessary to determine whether the glands are associated with cuticle hardening and maintenance, or whether they are associated specifically with reproduction.

The purpose of the present study was thus to further investigate the function of the tegumental glands of the male first gonopod in snow crab, *C. opilio*. The approach was twofold: more detailed histological and histochemical work to better document the gland-ejaculatory canal relationship and the nature of the gland secretions, and a developmental study to determine whether the degree of gland development is related to carapace hardening and consolidation (moult frequency inversely proportional to age) or to sexual maturity.

## MATERIALS AND METHODS

### Specimens and Sampling

A total of 17 male snow crabs were recovered from traps in the Baie des Chaleurs (New Brunswick, Canada), between November 1993 and November 1994, at depths of 60–120 m. Six crabs obtained in November 1993 were maintained until dissection in July 1994 in open-circuit holding tanks, supplied with water from the Baie des Chaleurs (mean temperature = 1°C, mean salinity = 29‰). The tanks were covered in order to simulate the extremely low light intensity of the crabs' natural habitat. They were fed *ad libitum* with shrimp or smelt once weekly, and detritus was removed the following day. Seven crabs were obtained in June 1994 and dissected the same week, while four others were obtained in November 1994, of which two were dissected the next day. The two remaining crabs were maintained as above and dissected in December 1994 (for lipid tests).

### Biometric Measurements and Maturity Categories

The maximum carapace width (CW) and cheliped height (CH) of all crabs were measured to the nearest millimeter using Vernier calipers. The CW:CH ratio was calculated and compared to a previously established scale in order to distinguish between morphometrically mature and immature crabs (Conan and Comeau 1986).

In order to quantify gland system development, the morphometrically immature crabs were further subdivided into 3 size groups, based on carapace width: small immature (SI: <40 mm), medium immature (MI: 40–70 mm), and large immature (LI: 70–100 mm). In order to compare homogeneous groups rather than a continuum of sizes, variation in specimen size within each group was minimized. The actual means and ranges were: SI 27.3–30.6,  $\bar{X}$  = 28.8; MI 53.6–66.0,  $\bar{X}$  = 61.1; LI 88.2–99.5,  $\bar{X}$  = 95.4.

### Dissection, Fixation, Histological Processing

The first gonopods of each crab were removed at the base of the protopodite and fixed as described below. The vas deferens was also removed and fixed, in order to effect whichever histological tests were found positive in the gonopod glands. The purpose of this procedure was to attempt to establish whether any secretions of the glands which might be transferred to the spermatheca during copulation could be confused with secretions from the vas deferens.

Prior experience had shown the first gonopods to be particularly problematic for micotome sectioning, due to the considerable difference in hardness between the cuticle and the very loose internal tissue. In an attempt to alleviate this problem, 3 fixation solutions were tested: Helly's, Böhm Strenger, and aqueous Bouin's (Vacca 1985). Of these, only the aqueous Bouin's al-

lowed sectioning. Penetration of the fixative was enhanced by first bisecting the gonopod at the distal extremity of the pinnate setae distribution, and then piercing the cuticle on the face opposite to the ejaculatory canal with entomological needles. The fixation was then performed under vacuum, in order to eliminate air from the ejaculatory canal which had been introduced during dissection. Despite these precautions, the internal tissue was often separated from its points of attachment to the cuticle during sectioning. The best sections were obtained from gonopods prepared as above and left for 3 months in aqueous Bouin's fixative. No improvement was obtained from resin-embedded gonopods, which presented incomplete matrix penetration.

One gonopod each from a mature and large immature crab were fixed in calcium-formol (Vacca 1985) for lipid histochemistry.

All gonopods except those tested for lipids were dehydrated in an ascending ethanol-xylene series and embedded in paraffin. Serial sections were performed on the first 10 embedded gonopods, in order to determine the exact distribution of the glands. The 5 remaining paraffin-embedded gonopods were serially sectioned at 10  $\mu$ m within this region. Gonopods tested for lipids were frozen on cryotome stubs and cryosectioned at 16  $\mu$ m within this region.

### Histochemistry

The histochemical tests performed on the gonopod sections are indicated in Table 1. Due to the non-specific nature of the Orange G test (all aminated substances were targeted), no negative control was used in this case only. The tests were chosen to cover the major groups of biochemical constituents likely to be either secreted or stored. Staining times were: alcian blue, 30 min; Schiff, 1 sec (Fisher Scientific) or 10 min (BDH); Sudan black, 10 min; Nile blue 15 min. Alcian blue was contrasted either with trioxymethatein (Gabe 1968) or nuclear fast red (Vacca 1985). A combined alcian blue/periodic acid-Schiff staining procedure was performed in order to identify glands containing a mixture of acid and neutral mucopolysaccharides; staining times were as above, with alcian blue preceding Schiff reagent. Due to the extremely small size of gonopods from the small and medium immature size groups (approx. 8–9 mm long, glandular region approx. 1 mm), cryostat sectioning for lipid tests could only be performed on mature and large immature individuals.

### Stereology

Stereological counts were performed on gonopod sections of 3 males from each maturity category. A 540-point matrix (each point separated by 1 cm) was superimposed on a video monitor screen output from the microscope. Counts were performed at 40 $\times$  for all categories except small immatures, which were done at 100 $\times$ . The volume fraction of the gonopod occupied by glands (gland volume fraction) was determined for 3 complete gonopod

TABLE 1.

*Chionoectes opilio*. Histochemical tests performed on gonopod tegumental glands.

Substance	Test	Positive Control	Negative Control	Reference
Lipids	Sudan black Nile blue	Hepatopancreas	Lipid extraction	High 1984
Proteins	Orange G	Muscle	—	James and Tas 1984
Acid MPS	Alcian blue	Hepatopancreas	Amylase digestion	Vacca 1985
Neutral MPS	PAS	Hepatopancreas	Amylase digestion	Vacca 1985

sections for each individual, and the individual mean was then calculated (Beninger 1987). The mean gland volume fraction and standard deviation were then calculated from the individual means of the 3 crabs of each maturity category.

## RESULTS

### *Gland Distribution, Aspect, and Histochemical Features*

Serial sections of gonopods from all size categories showed the glands to be restricted to a specific region at the base of the endopodite; this distribution is not characterized by any particular external anatomical markers (Fig. 1). The glands were of the rosette type (Talbot and Demers 1993), and were present to varying degrees in all crabs examined (Fig. 2), with the exception of the smallest immature individual. The glands were fewest in the small immature category; however, cellular aggregates visible as local concentrations of nuclei which may have been gland anlagen were observed using nuclear red counterstain in both the small and medium immature categories. Glands were separated by loose

connective tissue. Glands contained either acid or neutral mucopolysaccharides, or a mixture of both types of mucopolysaccharide. The glands were negative for lipid, and slightly positive for aminated substances (consistent with the positive mucopolysaccharide results). The vas deferens contained a mixture of acid and neutral mucopolysaccharides. The neutral MPS were visible as numerous red spheres in the surrounding acid MPS, contrasting with the mixed MPS in the gonopod glands, which had a more homogeneous appearance.

With the exception of the smallest immature crab (which had no glands, as noted above) the glands were grouped around the ejaculatory canal, while the gonopod muscles were situated opposite the ejaculatory canal. In mature, large and medium immature categories, the ejaculatory canal cuticle possessed pores, which contained substances exhibiting the same histochemical properties and microscopic appearance as the glands (Fig. 2.3, 2.4). The glands were connected to the pores via a network of ducts (Fig. 2.2, 2.3, and 2.4); no pores or ducts were present in the cuticle outside of the ejaculatory canal. Distinct dilatations of the ducts were observed on the proximal face of the cuticle. In the small immature category, no pores or dilatations were observed in histological sections of the cuticle, even in crabs which presented glands.

### *Stereology*

The mean gonopod gland volume fractions for each maturity category are presented in Figure 3. The lowest volume fraction was found in the small immature category; there was a fivefold increase between the small immature and medium immature categories, and the volume fraction continued to increase in the subsequent maturity categories. The gland volume fraction of the mature crabs was an order of magnitude larger than that of the small immature category (8% vs. 0.8%).

## DISCUSSION

### *Gonopod Tegumental Glands as Accessory Reproductive Glands*

Taken together, the results of the present study are congruent with a reproductive function for the gonopod tegumental glands in *C. opilio*. As was previously demonstrated in adult snow crabs (Beninger et al. 1991), the duct system and associated cuticular pores are directed exclusively toward the ejaculatory canal in all but the most immature individuals (small immature size group), which lacked pores and had very few glands. The absence of such pores in these individuals, and in the rest of the cuticle of all other specimens, is inconsistent with the competing hypothesis of cuticle maintenance or hardening. Finally, the direct relation between volume fraction of the glands and maturity category is unequivocal; it is antithetical to functions associated with cuticle hardening or maintenance, since younger animals moult more frequently than older ones and hence would require the opposite volume fraction-maturity relationship.

### *Ontogeny of Gonopod Gland System*

Cell aggregates observed in the small and medium immature individuals may represent gland anlagen, as reported for *Callinectes sapidus* (Johnson 1980). The glands differentiate, become more numerous, and the associated duct system develops as the individual grows. Detailed ultrastructural investigation would be

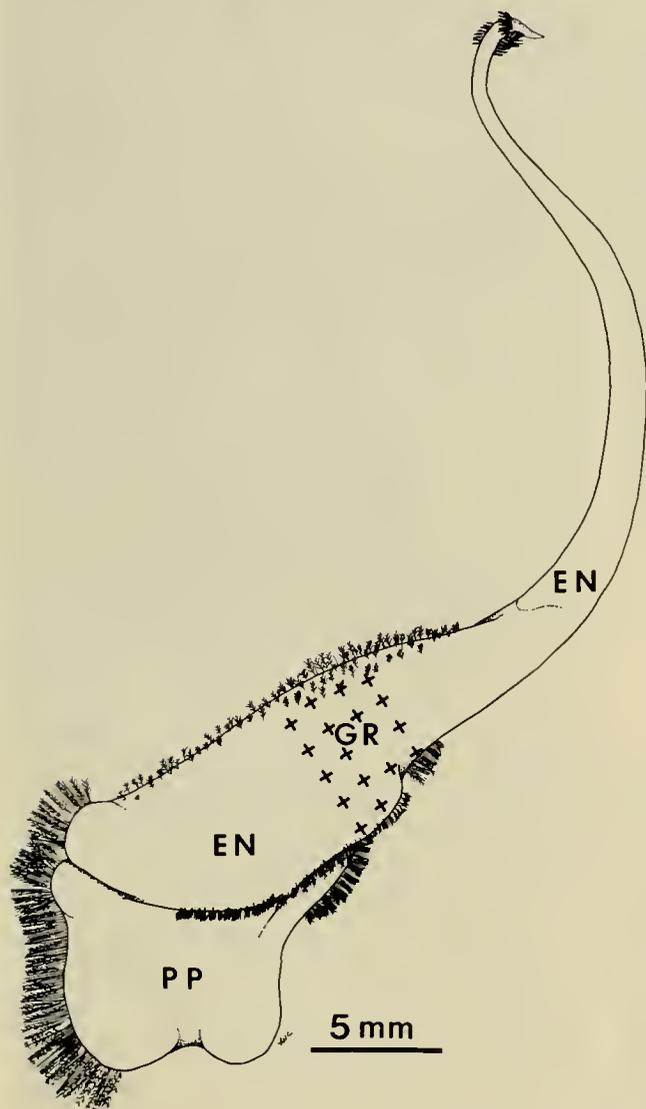


Figure 1. General morphology of mature *Chionoecetes opilio* first gonopod. EN, endopodite; GR, gland region; PP, protopodite.

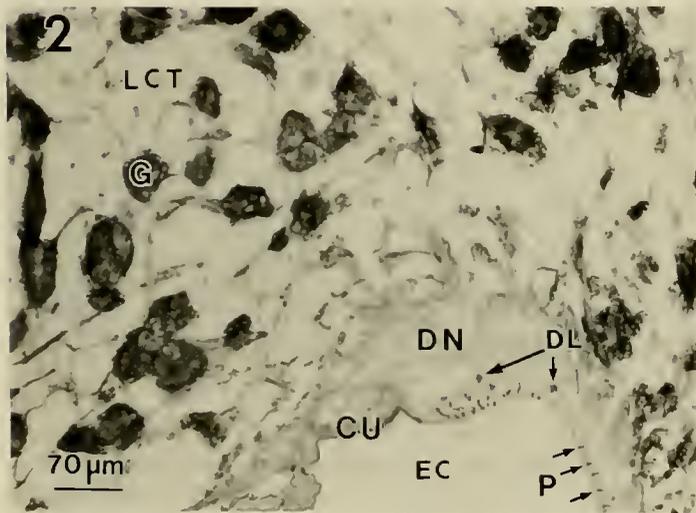
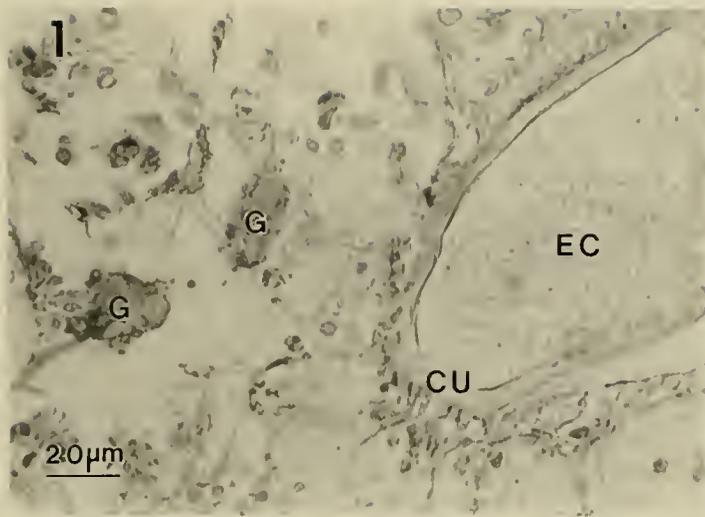


TABLE 2.

*Chionoectes opilio*. Results of histochemical tests performed on gonopod tegumental glands.

Category	Substances Tested			
	Lipids	Acid MPS	Neutral MPS	Aminated Substances
Mature Large	—	+++	++	+
immature	—	++	++	+
Medium immature	*	++	++	+
Small immature	*	++	++	+

—, negative; +, weakly positive; ++, positive; +++, strongly positive; \*, test not possible.

most appropriate, in order to fully elucidate the development and constitution of these glands.

The continuity of the gland secretions with the surface of the ejaculatory canal cuticle is accomplished via the cuticular pores. Histologically, their structure resembles that of the cuticular pores characteristic of the general crustacean exoskeleton (Halcrow 1993), with the notable exception of their much larger dimension (2–4  $\mu\text{m}$  vs 0.1–0.2  $\mu\text{m}$ ). This large pore dimension is consistent with a function in rapid secretion of large amounts of fluid. The cuticular pores appear only after the first glands and duct system have differentiated, several instars after metamorphosis, again underscoring the link with reproduction rather than cuticle hardening or maintenance.

The sampling carried out in the present study precluded observations of a seasonal nature, in the event of seasonal cycles in gland contents. Although highly desirable, such sampling is extremely difficult for the population studied, due to weather conditions and ice cover from November through May. Moreover, the stereological results clearly demonstrate a close relationship between maturity and gland volume fraction, regardless of sampling date. We would expect seasonal differences, if present, to consist of variations in the amount and type of secretions in the glands. Studies now in progress address these questions in relation to the adult reproductive cycle in more accessible populations and in other crab taxa.

#### Gonopod Tegumental Glands and Maturity Criteria

Maturity in male snow crabs may involve several aspects: morphometric, physiological, and functional (Conan and Comeau 1986, Comeau and Conan 1989, Claxton et al. 1994). There is disagreement, however, regarding the means of assessing maturity; this problem is related to and compounded by the lack of consensus on the existence of a male terminal moult in this genus,

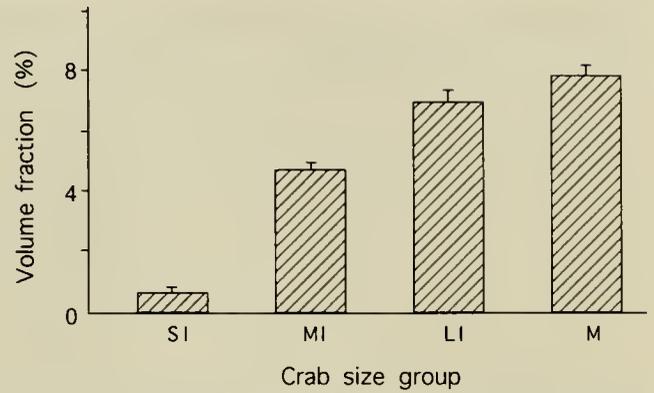


Figure 3. Volume fraction ( $\bar{x} \pm$  standard deviation) of tegumental glands within histological sections of gonopods of crabs from each size group. SI, small immature; MI, medium immature; LI large immature; M, mature.

and whether the maturity moult is the terminal moult (Dawe et al. 1991, Paul 1992, Sainte-Marie and Hazel 1992). The stereological results of the present study show that gonopod tegumental glands continue to develop through all size groups to the morphometrically mature crabs. A stereological study involving a statistically appropriate number of individuals might usefully be undertaken, in order to determine whether the volume fraction of the gonopod tegumental glands could be used as a maturity criterion in this debate, in conjunction with the present morphometric technique and vas deferens weights (Comeau and Conan 1989).

#### Possible Roles of the Gonopod Glands

The histochemical data of the present study show that the main, if not exclusive, constituents of the secretions are mucopolysaccharides. The foremost biological properties of mucopolysaccharides are related to their viscosities. The *C. opilio* gonopod gland system comprises glands with exclusively neutral secretions, exclusively acid secretions, and mixed secretions. Increasing mucus viscosity is associated with a corresponding increase in the proportion of acid mucopolysaccharides (Grenon and Walker 1980, Moreno et al. 1982); this is supported by visual observations of mucus behaviour on surfaces known to secrete specific MPS types (Beninger et al. 1992, 1993). Neutral mucopolysaccharides are thus very fluid, and they may be used to reduce the viscosity of more highly viscous mucopolysaccharides (St-Jean 1993). The matrix of the vas deferens is composed of poorly mixed acid and neutral mucopolysaccharides, and is thus of intermediate viscosity. The seminal secretions and spermatophores must be forced through the narrow ejaculatory canal (diameter approx. 50  $\mu\text{m}$ ) (Beninger et al. 1991) during copulation; hence a reduction in their viscosity would facilitate spermatophore transfer, and may even be a necessary condition for such transfer. Such a process would

Figure 2. Histological sections of *Chionoectes opilio* first gonopod. Alcian blue - PAS stain. 2.1. Small immature individual (CW = 28.5 mm). Note paucity of glands (G) and absence of pores in cuticle (CU) of ejaculatory canal (EC). 2.2. Medium immature individual (CW = 53.6 mm). Note increased density of glands (G) compared to preceding small immature specimen (ratio of 2.1:1 compared to Figure 2.1, after standardization of surface areas). DN, extensive duct network; P, cuticular pores, often accompanied by proximal dilations (DL); LCT, loose connective tissue. 2.3. Detail of cuticle (CU) and duct network (DN) of same individual. Note penetration of pores (P) into cuticle, and granular substances within pores, similar to that in duct network (arrowheads). 2.4. Composite micrograph detail of same individual, showing pathway from gland (G) via duct (D) to duct network (DN) and cuticular pores (P). Note proximal dilatation (DL) of pore, and presence of granular substances at all levels of pathway (arrowheads). CU, cuticle.

require that the neutral mucopolysaccharide-containing glands be capable of independent secretion.

The presence of a distinct layer of acid mucopolysaccharides has been reported in the ventral region of the spermatheca of copulated female snow crabs; it was suggested that this feature might originate with the gonopod gland system (Beninger et al. 1993). The results of the present study are consistent with this hypothesis only insofar as the acid mucopolysaccharide-containing glands would also be capable of independent secretion. Besides their viscous properties, acid mucopolysaccharides have been shown to possess bacteriostatic properties, and may function to this effect in 2 species of penaeid shrimp (Sasikala and Subramoniam 1987). These secretions may thus act to protect the transferred spermatophores within the spermatheca from opportunistic microbes. Further research is needed to verify this hypothesis.

Mixed-secretion, intermediate viscosity mucopolysaccharides are ideal for lubrication. The specific distribution of the gland

region within the first gonopod corresponds to the insertion of the second gonopod, which acts as a piston to force seminal fluids from the penis along the ejaculatory canal of the first gonopod (Beninger et al. 1991); lubrication would facilitate this movement and reduce the risk of damage to the cuticle of the ejaculatory canal. The above-mentioned functional possibilities are not mutually exclusive, but do imply some degree of differential control. Future studies might explore these avenues.

#### ACKNOWLEDGEMENTS

The authors wish to thank Mad. Chantal Gionet for her skilled technical assistance, as well as M. L. Blanchard for his excellent photographic work. Funding for this study was provided by operating grants to PGB from the Natural Sciences and Engineering Research Council and the Faculté d'études supérieures et de la recherche de l'Université de Moncton.

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## FIELD STUDIES OF THE REPRODUCTIVE BIOLOGY OF THE SPINY LOBSTERS *PANULIRUS ARGUS* (LATREILLE) AND *P. GUTTATUS* (LATREILLE) AT BERMUDA

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**ABSTRACT** A variant on the standard leg length assessment of the size at first physical maturity is described and applied to *Panulirus argus* and *Panulirus guttatus* at Bermuda. This is based on the relative length of the carapace and the longest segment of the second walking leg. Differential growth of this leg joint (meropodite) relative to the carapace is observed at maturity. The size at which maturity occurs is determined by intercept analysis of plots of meropodite length on carapace length (CL). For female *P. argus* the ratio changes in a negative direction at maturity. For male *P. argus*, the reverse occurs. For both female and male *P. guttatus* the ratio changes in a negative direction, but to a greater degree in females. Using the technique, first physical maturity for *P. argus* females captured at Bermuda in 1986 and 1987 was measured to be 86.0 mm carapace length, CL (standard deviation 5.1 mm CL), and for males was 97.4 mm CL (sd 5.0 mm CL). For *P. guttatus*, the comparative data were 59 mm (sd 4 mm CL) for females and 69.3 mm (sd 1.7 mm CL) for males. Field observations of trappable females bearing eggs and/or spermatophores confirmed these values. Observations on sex ratio and distribution by depth of *P. guttatus* are described. Evidence of an inward migration of *P. guttatus* lobsters from the outer terraces and slopes to the reef-crest is presented.

**KEY WORDS:** Size at maturity, spiny lobsters, *Panulirus argus*, *Panulirus guttatus*, breeding-migration, sex ratio

### INTRODUCTION

Size at maturity (SAM) in Spiny Lobsters has 2 components; physiological maturity (when the gonads become capable of producing viable gametes) and functional maturity (when all secondary characteristics have developed to an extent which allows successful mating). A problem, as far as fishery management is concerned, is that populations of lobsters reach functional maturity at different sizes. Thus, Sutcliffe (1952) estimated that 50% of females of the Bermudan Spiny Lobster, *Panulirus argus* (Latreille) were physically mature at a carapace length (CL) of 90 mm, whereas those from the Lower Florida Keys reach physical maturity at 80 mm CL (Warner 1977).

Various factors may influence size at maturity including fishing pressure, density dependent growth, food availability and quality (Kanciruk 1980). Water temperature too may be important (Chittleborough 1976). Consequently animals reaching maturity at a similar age are not necessarily of the same size.

Complicating the issue of determining SAM still further are the variety of criteria which have been used. As Kanciruk (1980) notes, the definitions of a mature female lobster include: the smallest female observed with ripe ovaries; the smallest carrying a spermatophore; the smallest carrying eggs; the size class of female most frequently found to be carrying eggs; the mode of all females with eggs or with spermatophores; and the size class possessing specific female external characters.

Male Spiny Lobsters use their front walking legs in the mating act and in dominance displays (Berry 1970) and it has long been known that in various species the adult males show substantial development of the walking legs relative to carapace length (Craw-

ford and De Smidt 1922; Kubo 1938, *Panulirus japonicus* v. Siebold; Gordon 1960, *Panulirus ornatus* Fabricius; and Berry 1970, *Panulirus homarus* Linnaeus).

Following on from these findings, George and Morgan (1979) have investigated size at first physical maturity (SAFPM) in male and female *Panulirus versicolor* (Latreille) by analysis of the relationship between overall leg length, for the different walking legs, and carapace length. They observed that the length of each of the first 3 walking legs (especially the second and third) increases relative to the carapace when the males reach a certain size. Conversely the length of each of the 5 walking legs of the females (and especially that of the fifth leg) decreases when the individuals reach a specific size. These changes in relative leg length are considered to relate to the onset of maturity (George and Morgan 1979).

Extending these earlier studies, the principal aim of the present studies was to estimate the size at first physical maturity (SAFPM) of male and female *P. argus* and *P. guttatus* at Bermuda using a variant of the leg-length technique; in part for comparison with the findings of Sutcliffe (1952). Consideration is also given to the estimation of size at maturity of the females of the 2 species using the criterion of the size at which 50% of the individuals show signs of sexual activity (i.e., bear eggs or spermatophores). Observations have also been made with regards to some little known aspects of the reproductive biology of *P. guttatus*, including migration, sex ratio and distribution by depth.

### MATERIALS AND METHODS

All field studies were undertaken from Bermuda Biological Station for Research, on the Bermuda Platform and Argus Bank, Bermuda (Fig. 1).

#### Animals

#### Juveniles

Most of the juvenile of *P. argus* used were sampled from casitas (scaled down lobster shelters) set out in inshore waters at

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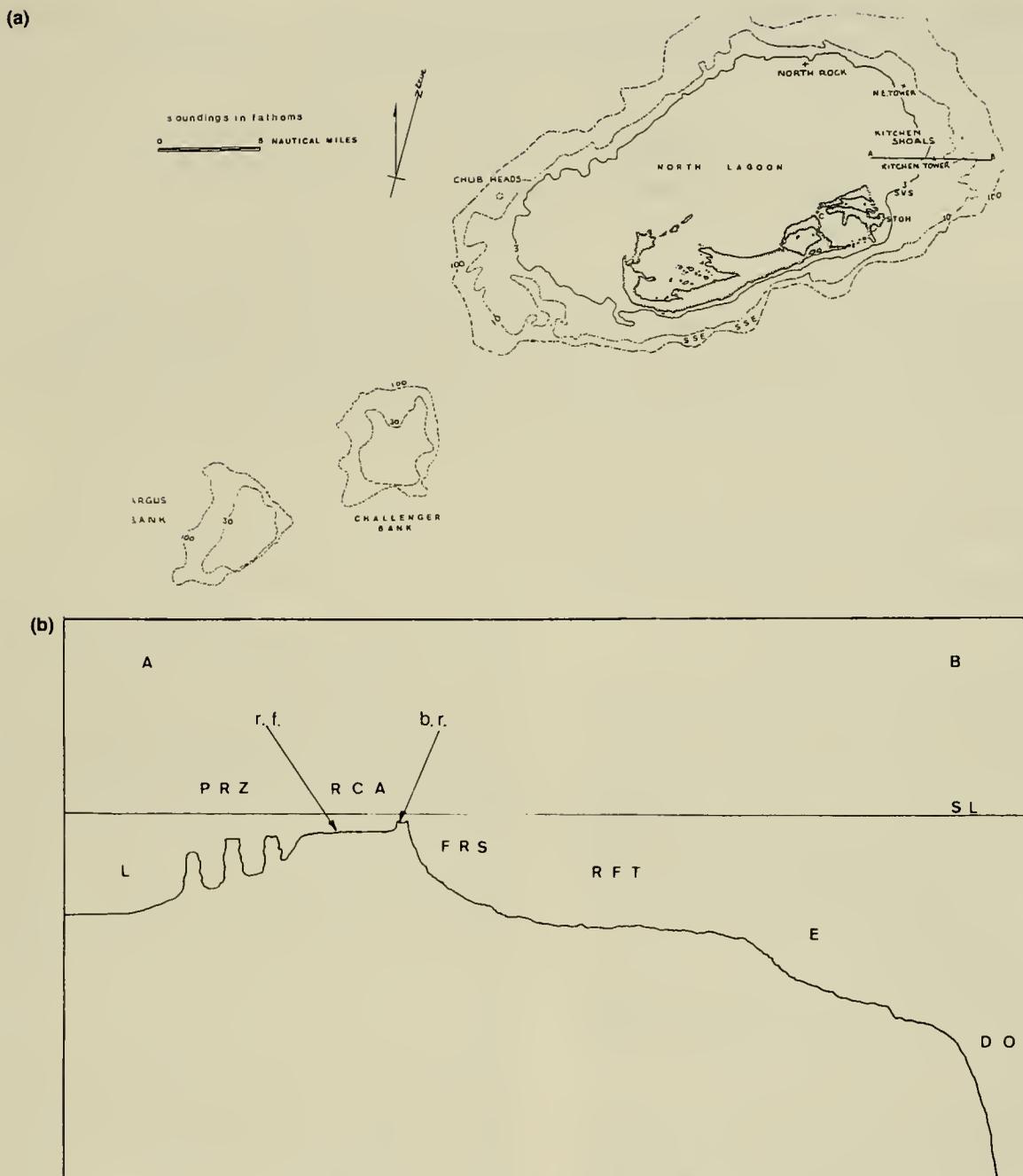


Figure 1. The transect trap line, study areas and reef profile. (a) The transect and study areas. The position of the west to east trapping transect through Kitchen Tower is marked by the line AB. The locations of the trapping study areas are given by the following: Kitchen Tower (Kitchen Shoals); the East End Edge (located at the eastern end of the transect line); the northeastern rim reefs, terraces and slopes from North Rock to N.E. Tower to Kitchen Tower; the southern Platform edge (South Shore Edge, marked by SSE); St. David's Head (STDH); Sea Venture Shoals (SVS); the southwestern rim reefs, terraces and slopes (associated with Chub Heads); and Argus Bank, to the southwest of Challenger Bank. The location of the casita cluster in the inshore water system at the northeast of the island is given by C. (b) The reef profile. Sectional diagram showing the major physiographic provinces of the Bermuda Reef System. The profile shown is the section through A to B (a). L = Lagoon. SL = Sea level. PRZ = Patch reef zone in the lagoon, landward of the atoll-rim, of 6–12 metres depth (3–6 fathoms). RCA = Reef Crest Area comprising the reef flats (rf) and the algal cup reefs or 'boiler reefs' (br) which break the surface, forming the atoll-rim; the reef flats are located at ca. 6–8 metres depth (3–4 fathoms). FRS = Fore-reef slope, sloping reef surface seaward of the reef-crest or 'breaker-line' ranging from ca. 12–18 metres depth (6–9 fathoms). RFT = Reef-front terrace or 'main terrace' at 18 metres (10 fathoms). E = Edge of the island shelf, or 'platform edge', i.e., the terraces and slopes seaward of the reef-front terrace out to the drop-off; in this province, *P. guttatus* was only found at 22 to 45 metres depth (11 to 22 fathoms). D.O. = The 'drop-off', i.e., the beginning of the steep, precipitous slope to the ocean floor, at ca. 68–78 metres depth (35–40 fathoms).



Figure 2. Measurement of an index of leg length in spiny lobsters. The ventral view of the longest segment of the second walking leg is shown. The measurement taken was the distance between X and Y. X is the middle of the distal joint of the longest segment, and is marked by a thin grey line of gelatinous tissue. Y is the most distal point of the proximal joint of the longest segment.

2.9 to 3.3 m depth in the way described by Evans et al. (1994). A few additional specimens were supplied by fishermen.

#### Adults

Adults of both *P. argus* and *P. guttatus* were obtained by trapping with traditional Bermudan arrowhead traps with a 4.1 mm (hexagonal) mesh (Sutcliffe 1952) set on a transit line (A—B) across the North Eastern reef system (Fig. 1). Traps were baited with fish waste and set in sand-floored depressions (white holes) in the reef. The overall transect length was 7 miles, with trap spacing of 0.5 miles. Trapping was undertaken from May to October 1987. Sampling was twice per week. Additionally, lobsters were taken from Sea Venture Shoals and from the outer terraces and slopes in the same general area and from the outer reefs off the South Shore (Fig. 1). A number of specimens taken by commercial vessels from Argus Bank were also measured.

#### Measurements

##### Carapace Length

CL measurements were determined with a SPI vernier type calibre 6914 calliper to the nearest 0.1 mm. Some potential for error is inevitable but successive measurements on the same individual suggest that this will not be larger than 0.5 mm even for lobsters with a CL of 100 mm or more and less for smaller specimens.

##### Meropodite

Precise measurements of the whole walking leg are difficult to make because of the limb joints. In the present study measurements were therefore restricted to the meropodite (the longest segment of the limb) of the second walking leg (distance X—Y) (Fig.

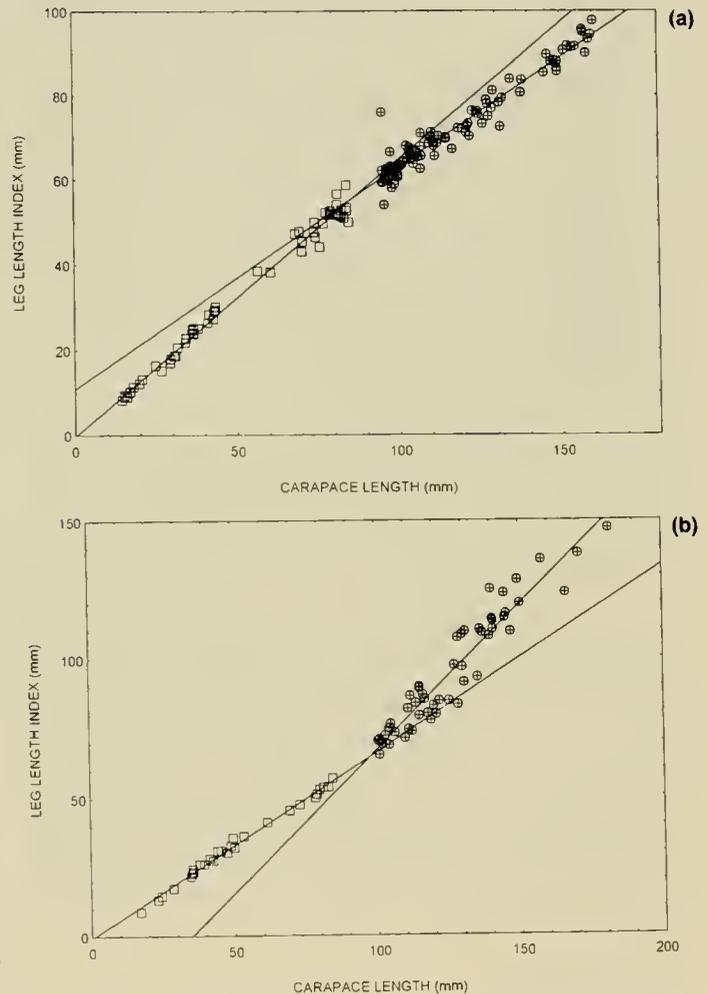


Figure 3. LLI/CL intersect analyses for *P. argus* size at first physical maturity, Bermuda. The graphs show the estimated size at first physical maturity for (a) females (86.0 mm CL) and (b) males (97.4 mm CL), based upon linear regression of leg length index (LLI) on carapace length (CL). In (a), the points for carapace lengths <85.0 mm are shown as squares and those for carapace lengths >94.9 mm are shown as circles with cross-hairs ( $n_1 = 59$ ;  $n_2 = 92$ ). The respective linear regression lines:

$$y = +0.650x - 0.242 \quad (1)$$

$$y = +0.521x + 10.851 \quad (2)$$

Solution to derive the coordinates of the intersection point is possible, by simultaneous equations. Subtracting Eqn 2 from Eqn 1,  $x = 86.0$  mm CL. In (b), the points for carapace lengths <85.0 mm are shown as squares and those for carapace lengths >99.9 mm are shown as circles with cross-hairs ( $n_3 = 51$ ;  $n_4 = 28$ ). The respective linear regression lines:

$$y = +1.038x - 36.405 \quad (3)$$

$$y = +0.672x - 0.739 \quad (4)$$

Simultaneous equation solution (3-4):  $x = 97.4$  mm CL.

TABLE 1a.

Female size distribution and observations of evidence of reproductive activity in *P. argus*; results from trapping the southern edge of the island shelf, 1986.<sup>a</sup>

CL Class (mm)	No. Without Sign	No. With Sign
65-69.9	0	0
70-74.9	1	2
75-79.9	2	3
80-84.9	23	12
85-89.9	30	36
90-94.9	27	37
95-99.9	4	17
100-104.9	9	11
105-109.9	6	2
110-114.9	1	5
115-119.9	0	1
120-124.9	0	2
125-129.9	0	1
130-134.9	0	1

2). X was taken as the mid point of the distal joint of the meropodite and Y as the middle of the proximal joint of the segment. The distance is referred to as the Leg length index (LLI).

#### Graphical Representation

By utilising the allometric growth of the meropodite relative to carapace growth it is possible to derive a carapace length which represents the presumptive age of sexual maturity for the population. This is taken as being the point at which the slope of the plot of LLI against CL changes slope. Points falling within a narrow band either side of the estimated intersection point were excluded from the linear regression analysis if they could not clearly be ascribed a relationship for regression. George and Morgan (1979) used this method for determining the size at first physical maturity of *P. versicolor*, but by the more difficult measurement of the length of the entire leg.

#### Statistical Treatment

Estimates of the x-value of the intercept, i.e., the mean x estimate for the intersection were made by 2 methods (1) by solution for x of simultaneous equations (of the 2 regression functions) and (2) using the standard formula  $x = (b1 - b2)/(a2 - a1)$ . Estimates of variance about this mean (from which confidence intervals may be estimated if required for comparison) were made by the Delta Method (as outlined in Agresti 1990). ( $b1$ ,  $b2$ ,  $a1$  and  $a2$  are the respective slopes and intercepts of 2 linear regression lines of the form  $y = a.x + b$ ). The methods of estimation of the mean x estimate gave very similar results.

### RESULTS

#### *P. argus*

##### Size and Leg-Length Index

Figure 3a illustrates a scattergram of leg-length index against carapace length for mature and immature female *P. argus*. The graph indicates that larger individuals have a relatively smaller

value for the ratio LLI/CL than do juveniles. Intersection of the regression lines occurs at a carapace length of  $86.0 \pm 5.1$  mm (standard deviation from the Delta Method as detailed in Agresti 1990). (The results by the 2 methods were (1) simultaneous equations,  $x = 86.0$  mm CL; (2)  $(b1 - b2)/(a2 - a1)$ ,  $x = 85.992$  mm CL). Solution by simultaneous equations is given in Appendix 1.

By contrast a scattergram of LLI against CI for males shows an intersection of regression lines at  $97.4 \pm 5.0$  but with the value of the ratio LLI/CL increasing with carapace length for the mature animals (Fig. 3b). (The results by the 2 methods were (1) 97.4 mm CL; (2) 97.448 mm CL).

Figures 3a,b also indicate that for juvenile animals the ratio LLI/CL is very similar for males and females. A similar observation has also been made on *P. versicolor* (George and Morgan 1979).

#### Indices of the Onset of Reproductive Activity

Comparison of carapace length in relation to signs of sexual activity were undertaken in the period late August to October 1986 along the South Shore Edge. The timing was late in the reproduc-

TABLE 1b.

Female size distribution and observations of evidence of reproductive activity in *P. argus*; results from trapping at the Northeastern, Northern and Southwestern rim reefs, reef-front terraces, and platform edges, 1987.<sup>b</sup>

CL Class (mm)	Number in Category			
	E	*	W	N
60-64.9	0	0	0	1
65-69.9	0	0	0	0
70-74.9	0	0	0	2
75-79.9	0	0	0	7
80-84.9	0	0	0	18
85-89.9	0	4	0	34
90-94.9	1	4	1	17
95-99.9	1	9	4	14
100-104.9	3	8	0	5
105-109.9	5	5	0	0
110-114.9	4	2	1	0
115-119.9	1	2	0	0
120-124.9	1	0	0	0

<sup>a</sup> Trapping was at a depth of approximately 58 metres (30 fathoms), from late August to early October 1986, along the South Shore Edge, after the height of the breeding season. The modal carapace length (CL) of females was 88 mm. Most females had already shed their eggs, so evidence of reproductive activity was taken as presence of spermatophores (whole or eroded). The number in the total sample was 233.

<sup>b</sup> Trapping was carried out in depths of 6-66 metres (3-34 fathoms) June to October and included a sample derived from the transect across the northeastern reefs from 6-35 metres depth (3-18 fathoms). The total sample is 154.

Key:

E = egg-bearing (or bearing a remnant of eggs).

\* = bearing eroded spermatophores.

W = bearing 'whole' (i.e. intact) spermatophores.

N = bearing no sign of reproductive activity.

tive season and most of the reproductively active females had already shed their eggs. The criterion for reproductive activity was therefore taken as being the presence of a whole or eroded spermatophore. The results for 233 individuals are presented (Table 1a). In Figure 4a these data are plotted as a line graph of percentage of class showing signs of sexual activity against the median of the class size. Thirty six out of sixty six females (55%) were reproductively active in the 85–89.9 mm CL size class. Size classes below and above this range showed a progression from 34 to 58%. The results suggest that the size at which 50% of females are functionally mature is close to 86 mm.

By contrast with these animals from the South Shore edge, examination of a further 140 individuals from the North Eastern, Northern and South Western reef systems between June and October 1987 gave a rather different result (Table 1b, Fig. 4b). Here the data imply that it is only at a CL of 99 mm that 50% of the cohort are reproductively active.

It is recognised that the numbers on which these estimates are

TABLE 2.

Tabulations for estimation of the size at first physical maturity of female *P. guttatus* lobsters.<sup>a</sup>

(1) St. David's Head, 1986			
Date Female Captured	Size CL, mm	Evidence of Reproductive Activity	
27 August	61.5	egg-bearing	
27 August	62	no sign	
3 September	42.5 <sup>b</sup>	no sign	
8 September	68	no sign	
16 September	55.5	egg-bearing	
16 September	65	no sign	
16 September	56	egg-bearing	
23 September	61	eroded spermatophore	
3 October	59.5	no sign	
3 October	69	no sign	

(2) Northeastern Reef System, 1987				
Carapace Length Size Class, mm	Number of Females Captured, by Category of Reproductive Activity			
	E	*	W	N
50–54.9	1	0	0	1
55–59.9	7	2	0	6
60–64.9	2	2	0	3
65–69.9	1	0	0	1
70–74.9	0	0	0	2

<sup>a</sup> This presents the results of observations and measurements made at sea during experiments: (1) off St. David's Head at the seaward edge of the line of boiler reefs, i.e., near the reef crest, 21 August–3 October, 1986; and (2) across the northeastern rim reefs, terraces and slopes, 6–35 metres depth (3–18 fathoms) 7 May–21 October, 1987 (based on Table 4).

<sup>b</sup> Small-mesh traps were used at St. David's, and this single small female was caught, with 7 mm pleopodal setae. (Other females's setae measured 9–11 mm).

Key:

E = egg-bearing (or bearing a remnant of eggs).

\* = bearing eroded spermatophores.

W = bearing 'whole' (i.e., intact) spermatophores.

N = bearing no sign of reproductive activity.

based are rather small. Combination of the data was therefore undertaken to derive mean data for the whole Bermuda platform. The result (Fig. 4c) suggests that SAM lies close to 92.5 mm CL for female *P. argus*.

### *P. guttatus*

#### Size and Leg-Length Index

Figures 5a,b present plots of LLI against CL for the females and males of Guinea Chicks, *P. guttatus*. As for the female of *P. argus*, an alteration in the slope of the regression of the scattergram data occurs. In the females there is a decrease in the slope for the larger individuals (Fig. 5a). By contrast with the male of *P. argus* there is a negative change in the slope with size in *P. guttatus* males (Fig. 5b). The relative negative change of slope is greater for female *P. guttatus* than for male *P. guttatus*, however.

Regression lines for female *P. guttatus* intersect at  $59 \pm 4$  mm CL (standard deviation by the Delta Method) and those for males at  $69.3 \pm 1.73$  mm CL. The estimate of the intercept for female *P. guttatus* is quoted to less significant figures because, although the inflection in the curve is quite clear, the number of females measured was quite small ( $N = 52$ ).

#### Indicators of the Onset of Reproductive Activity

Munro (1974) found that *P. guttatus* females are infrequently caught by nets with a 4.1 mm mesh. Similarly, the numbers of females obtained in the present study was small.

Egg-bearing females were caught in the period 23rd May to 20th August, but later in the season (27th Aug to 24th September) only individuals with eroded spermatophores were caught (Table 2). No ovigerous females were taken in the winter or spring months (Evans and Lockwood 1994).

The percentage of reproductively active females found at Bermuda, i.e., those with eggs or spermatophores, for different size classes are presented in Figure 6. The numbers are small, particularly in the critical region, but suggest that the SAM (taken as the size class at which 50% are functionally mature) lies in the carapace length range 52.5 to 57.5 mm.

#### Range and Sex Ratio of *P. guttatus*

Trapping in the present study has been undertaken in all the physiographic provinces of the Bermuda Platform reef system, viz patch reefs inside the reef crest, the reef crest area (reef flats, algal cup reefs and boiler reefs) the fore reef slope, the reef front terrace and the platform edge (Fig. 1b).

No Guinea chick lobsters were found landward of the patch reef zone but they were taken from all the areas of the atoll reef system from 6 m (3 fathoms) down to the limit of sampling, 43 m (22 fathoms).

Only rarely were individuals of CL less than 48–50 mm caught by the standard, 4.1 mm mesh, traps. Further, deployment of seven traps with a 2.1 mm mesh just seaward of the reef crest at St David's Head from mid August to early October 1986 resulted in the capture of only one individual with a CL smaller than 50 mm (a 42.5 mm female). It appears therefore that neither male nor females of *P. guttatus* smaller than 48–50 mm CL are readily trappable.

For individuals larger than 50 mm CL there is a distinctive sex difference in the numbers trapped. Thus, of the 919 *P. guttatus* trapped in 1986, only 70 were females. This apparent preponder-

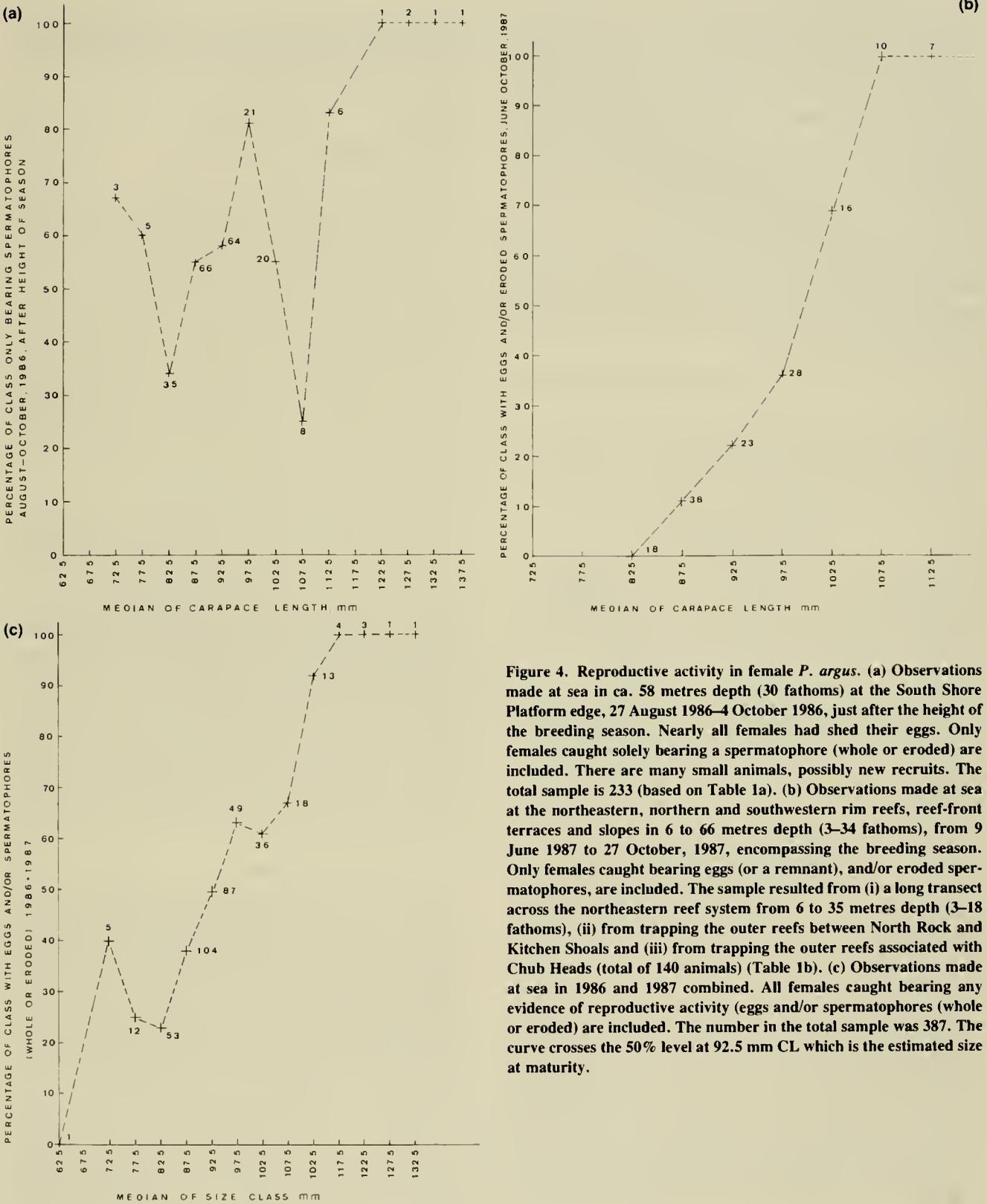


Figure 4. Reproductive activity in female *P. argus*. (a) Observations made at sea in ca. 58 metres depth (30 fathoms) at the South Shore Platform edge, 27 August 1986–4 October 1986, just after the height of the breeding season. Nearly all females had shed their eggs. Only females caught solely bearing a spermatoophore (whole or eroded) are included. There are many small animals, possibly new recruits. The total sample is 233 (based on Table 1a). (b) Observations made at sea at the northeastern, northern and southwestern rim reefs, reef-front terraces and slopes in 6 to 66 metres depth (3–34 fathoms), from 9 June 1987 to 27 October, 1987, encompassing the breeding season. Only females caught bearing eggs (or a remnant), and/or eroded spermatoophores, are included. The sample resulted from (i) a long transect across the northeastern reef system from 6 to 35 metres depth (3–18 fathoms), (ii) from trapping the outer reefs between North Rock and Kitchen Shoals and (iii) from trapping the outer reefs associated with Chub Heads (total of 140 animals) (Table 1b). (c) Observations made at sea in 1986 and 1987 combined. All females caught bearing any evidence of reproductive activity (eggs and/or spermatoophores (whole or eroded)) are included. The number in the total sample was 387. The curve crosses the 50% level at 92.5 mm CL which is the estimated size at maturity.

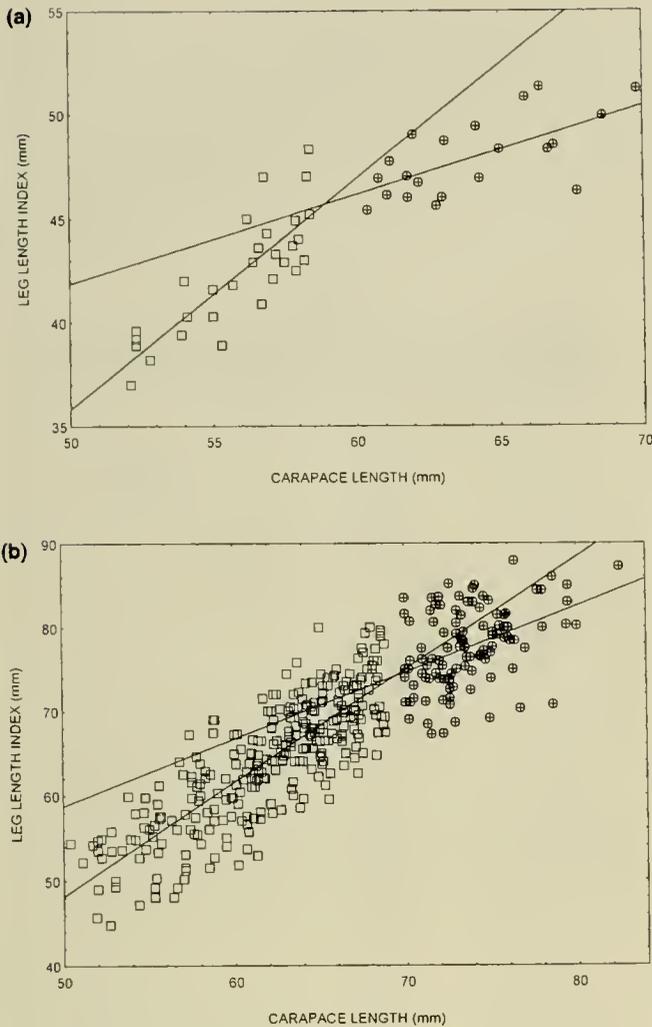


Figure 5. LL/CL intersect analyses for *P. guttatus* size at first physical maturity, Bermuda. The graphs show the estimated size at first physical maturity for (a) females (59.3 mm CL) and (b) males (69.3 mm CL), based upon linear regression of leg length index (LLI) on carapace length (CL). In (a), the points for carapace lengths <58.5 mm are shown as squares and those for carapace lengths >59.9 mm are shown as circles with cross-hairs ( $n_5 = 30$ ;  $n_6 = 22$ ). The respective linear regression lines:

$$y = +1.104x - 19.383 \quad (5)$$

$$y = +0.426x + 20.565 \quad (6)$$

Solution to derive the coordinates of the intersection point is possible, by simultaneous equations. Subtracting Eqn 6 from Eqn 5,  $x = 59.3$  mm CL. In (b), the points for carapace lengths <69.0 mm are shown as squares and those for carapace lengths >69.9 mm are shown as circles with cross-hairs ( $n_7 = 292$ ;  $n_8 = 108$ ). The respective linear regression lines:

$$y = +1.339x - 18.749 \quad (7)$$

$$y = +0.793x + 19.109 \quad (8)$$

Simultaneous equation solution (Equ. 7 - Equ. 8):  $x = 69.3$  mm CL. The change in slope of male *P. guttatus* is downward, which is the opposite to male *P. argus*. The degree of change is somewhat less, however, for male *P. guttatus* than for female *P. guttatus*. The change in slope of female *P. guttatus* is much greater than that of female *P. argus* (Fig. 3a). Size at first physical maturity of male *P. guttatus* is 10 mm CL greater than for females.

ance of males occurred in each of the reef provinces where traps were set (Table 3).

**Breeding Migration *P. guttatus***

The location (in terms of reef province) and reproductive state (in terms of egg remains, spermatophores and eroded spermatophores) of all females taken in 1987 from the transect area, Kitchen Shoals, Sea Venture Shoals and the Eastern Platform is given in Table 4. The data, for the period 7th May to 21st October 1987 suggest that there is an inward breeding migration of gravid females from the penultimate station near the eastern end of the transect towards the reef crest in the Kitchen Shoals area.

Evidence for a matching migration of males comes from records of monthly movement at the reef crest of the Kitchen Shoals area. Over the period May to September 1987 there was a distinct shift in the size composition (Fig. 7) from a preponderance of larger animals (which typically occur round the reef crest) to a majority of smaller animals more typical of deeper waters seaward of the reef crest (Evans 1988, 1989).

**DISCUSSION**

Size at maturity of the Bermuda Spiny Lobster, *P. argus*, was investigated by Sutcliffe (1952). He estimated that 50% of the females were physically mature at a carapace length of 90 mm.

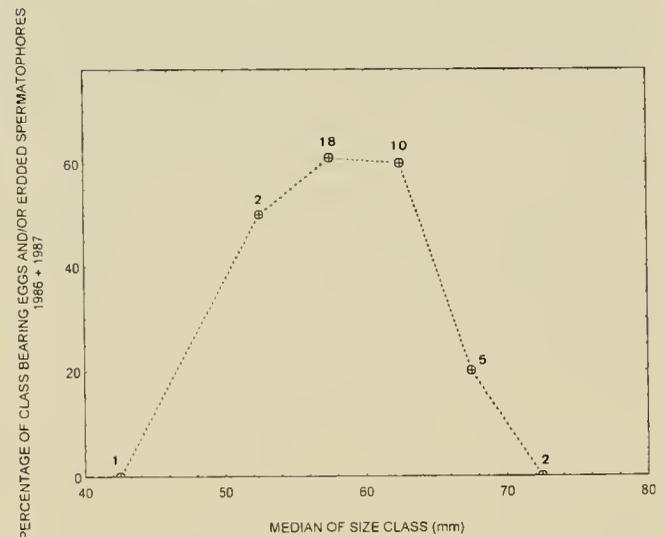


Figure 6. Field observations of evidence of reproductive activity in female *P. guttatus* lobsters. The graph shows the combined results of measurements of carapace length and observations of evidence of reproductive activity made at sea: (1) off St. David's Head, on the seaward edge of the line of boiler reefs; 21 August 1986-3 October 1986 (Table 2, part 1); and (2) on a transect across the northeastern reef system (patch reefs, rim reefs, reef-front terraces and slopes) 7 May 1987-21 October 1987 (Table 2, part 2). Evidence of reproductive activity was taken as presence of eggs (or a remnant of eggs) and/or eroded spermatophores. The x-values of the points are medians of 5 mm carapace length classes. The point at 42.5 mm CL refers to a single female, with relatively short (7 mm-long) pleopodal setae, caught whilst trapping off St. David's Head with small-mesh traps in the height of the breeding season in 1986. Sample sizes (for each class) are given alongside their respective points. It should be noted that 50% SAM is difficult to estimate with accuracy here because of the low sample sizes in the critical region, and the low total sample size ( $n = 38$ ), but it is suggested that it is in the region 52.5-57.5 mm CL.

TABLE 3.

Sex ratio of *P. guttatus* by physiographic province of the Bermuda reef system for the period May–October 1987 (from animals caught in transect pots).

Province Physiographic	No. Females	No. Males	SR
Patch Reef Zone	0	12	0
Reef Crest Area	4	63	6
Fore Reef Slope	1	10	9
Reef Front Terrace	2	89	2
Platform Edge	1	9	10

Sex ratio (SR) is the percentage of trap-caught females caught in the total sample (females + males).

Since then differences in the size of maturity of a lightly fished stock at Dry Tortugas with a more heavily exploited population in the lower Florida Keys (Warner 1977) have been interpreted as indicating an effect of fishing pressure. The question arises therefore as to whether exploitation of the Bermuda stock since Sutcliffe's 1952 study has influenced size at maturity.

In considering this issue due attention is necessary to the interpretation of the observations. In the present study 2 techniques have been utilised. The first involves comparison of the ratio of the length of the meropodite of the second walking leg to that of the carapace. This ratio changes dramatically (in a positive direction for the male and negatively for the female). This reflects the observations of Crawford and de Smidt (1922) with regard to the ratio of leg length to carapace length. The second method utilised direct observation of the proportion of trapped females carrying eggs and spermatophores or their remains. This method is comparable to that used previously at Bermuda (Sutcliffe 1952).

Using the ratio of meropodite length to carapace length as an indicator of sexual maturity is open to some potential sources of error: 1) The basic supposition is that the ratio of LLI/CL in relation to CL changes at maturity. Probably this reflects functional maturity in the female but functional maturity in the male is more likely to be dependent upon some degree of development of the secondary sexual characters, particularly the leg length. Potentially therefore in the male the point of inflection of a plot of LLI against carapace length may reflect the point at which physical maturity occurs but not necessarily functional maturity. 2) The determination of the true point of inflection is open to error. As pointed out by Kancirik (1980), a number of factors can affect the size of an individual at maturity. Close to the point of maturity therefore there may be some large individuals which are not mature and some smaller individuals which are mature. This would perhaps be immaterial if the whole population was being sampled. In a fished stock however unbiased sampling is not possible; in the lower size ranges there is escape from the traps whereas the upper sizes have been depleted by the fishery. A consequence of these 2 factors is that the data set tends to be bunched near the legal minimum size set for the fishery. This however, as noted above, is the region most likely to be affected by differences in the size at maturity. In an attempt to minimise the distortions imposed by such factors we have derived the point of intersection for *P. argus* using regression lines based on all data points except those for carapace lengths  $\pm 5$  mm either side of the point of intersection. This has the combined effect of removing some of the bias due to the bunching and some of the distortion consequent upon differential size at maturity. George and Morgan (1979) also applied this

technique to minimise distortion, though found it necessary to employ a somewhat larger interval.

Size at first physical maturity, SAFPM (as estimated by meropodite and carapace length measurements) is not strictly the same as size at maturity, SAM (estimated by the size at which 50% of females are functionally mature). As has been noted above, an individual may show all the physical signs of sexual maturity but not be ready to mate and/or produce eggs. The estimate of SAM

TABLE 4.

Observations on the reproductive biology of *P. guttatus*: Investigation of the breeding and migration of females, from a trapping transect across the northeastern reef system from 7 May 1987 to 21 October 1987.<sup>a</sup>

Date and P.E. Female Caught	C.L. (mm)	Category			
		E	*	W	N
7 May (RCA) <sup>b,c</sup>	58.6			×	
12 May (EE)	54.3			×	
23 May (EE)	55.5	×			
23 May (RCA)	72.8			×	
4 June (RFT)	56.3	×			
9 June (RCA)	72.8			×	
16 June (RFT)	53.2	×			
2 July (FRS)	55.7	×			
13 July (RCA)	57.7	×			
13 July (RCA)	57.8			×	
13 July (RCA)	62.4	×			
16 July (RCA)	66.6	×			
5 August (RCA)	56.2	×			
5 August (RCA)	61.0	×			
5 August (RCA)	58.3	×			
20 August (RCA)	57.6	×			
27 August (RCA)	63.6		×		
31 August (RFT)	56.0		×		
10 Sept. (RCA)	61.0			×	
14 Sept. (RCA)	60.4			×	
21 Sept. (RCA)	56.6		×		
21 Sept. (RCA)	57.5		×		
21 Sept. (RCA)	55.1	×			
24 Sept. (RCA)	63.4	×			
5 October (RCA)	60.0		×		
5 October (RCA)	68.3		×		
21 October (RCA)	58.2		×		
21 October (RCA)	57.9		×		

<sup>a</sup> Along the transect line (although the trap-rates were low), ovigerous *P. guttatus* occurred in a spatial and temporal sequence from near the shelf edge in May, at the reef-front terrace in June, at the fore-reef slope in early July, and at the reef-crest area from mid July to the latter part of August, suggesting an inward breeding migration of gravid females.

<sup>b</sup> The reef-crest area (RCA) captures listed above refer to the study area at Kitchen Shoals, except the female caught on 16th July, at Sea Venture Shoals study area.

<sup>c</sup> Key to Physiographic Environments (P.E.'s):

E = Shelf Edge at 22–35 metres depth (11–18 fathoms).

RFT = Reef-Front Terrace at ca. 18 metres (10 fathoms).

FRS = Fore-Reef Slope at 12–18 metres (6–9 fathoms).

RCA = Reef-Crest Area at 6–8 metres (3–4 fathoms).

Key to Categories:

E = egg-bearing (or bearing a remnant of eggs).

\* = bearing eroded spermatophores.

W = bearing 'whole' (i.e., intact) spermatophores.

N = bearing no sign of reproductive activity.

for female *P. argus* at Bermuda from the present study (92.5 mm CL, approx.) agrees with the estimate of 90 mm CL by Sutcliffe (1952), so it is unlikely that change in the rate of exploitation since 1952 has affected the mean size of female *P. argus* lobsters at maturity at Bermuda. This is supported by the finding of optimum exploitation rates for *P. argus* during the field work of the present study (Evans 1988, 1989).

A collation of the results on estimates of size at maturity in *P. argus* throughout its geographical range, sources from Morgan (1980) and Munro (1974) is presented in Table 5. The findings of the present study may be grouped with the first set of estimates in Table 5 (80–95 mm CL). It is perhaps relevant that all these areas have a relatively low water temperature compared to more southerly parts of the Gulf and Caribbean region. The other, second set of estimates, at 45–74 mm CL, is from areas where water temperature is higher. Morgan (1980) suggested a link between high water temperature and low size at maturity.

Weber in Belize (FAO 1968b) used the presence or absence of setae on the abdominal pleopods of a large sample ( $N > 720$ ) of females as an indicator of maturity, and on this basis, the smallest mature females were about 60 mm CL and full maturity was at 88 mm CL (mean size at maturity was 74 mm CL) (Table 5). In contrast, Munro (1974), in a very similar geographical location and latitude at Jamaica, found that the smallest female with eggs was of size 83 mm CL, and full maturity was attained at 110–119 mm CL. He concluded that 'the mean size at maturity in Jamaican waters might be about 95 mm CL' (Table 5). Munro found this incompatible with the Weber (FAO 1968b) findings. Over half of the 248 females examined by Munro (1974) at Jamaica were small (of  $< 80$  mm CL) but none carried eggs or 'spermathecae.' The 2 methods resulted in estimates of mean size at maturity which were

TABLE 5.

Collation of previous results on female size at maturity of *P. argus* by workers in the Gulf and Caribbean region.

Location	Female Size at Maturity (mm, CL)	Source
Antigua and Barbuda	80–90	(Peacock 1974)
Dry Tortugas, Florida	90	(Davis 1975)
Lower Florida Keys	80	(Warner 1977)
Jamaica	95	(Munro 1974)
Puerto Rico	80–90	(Feliciano 1958)
Bermuda	90	(Sutcliffe, 1952)
Bahamas	45	(Smith 1948)
British Honduras	54	(FAO 1968a:Allsopp)
	74	(FAO 1986b: Weber)
Cuba	57	(Buesa Mas 1974)
Venezuela	65	(Cobo de Barany 1972)

in disagreement with one another although the locations were very similar. The method Munro employed was a better technique as it was based upon evidence of reproductive activity rather than evidence of apparent ability to reproduce. However, in the present study, there was relatively much closer agreement of the results for female *P. argus* SAFPM by intersect analysis (86.0 mm, standard deviation 5.1 mm) and SAM (92.5 mm, approx.) by the measurement of carapace length and observation of evidence of reproductive activity. This suggests that the latter 2 methods are compatible and intersect analysis may be used to derive an indication of the mean size at maturity in Palinurid lobsters.

It is unclear why the meropodite of the second walking leg of male *P. guttatus* decreases in length relative to the carapace length at maturity. However, it is perhaps relevant that the change in the meropodite relative to the carapace at maturity in female *P. guttatus* is somewhat greater (difference in slope 0.674) than for male *P. guttatus* (difference in slope 0.546), and much greater than for female *P. argus* (difference in slope 0.129). Thus, meropodite length (LLI) does not increase relative to carapace length at maturity in every species of Palinurid lobster.

Berried female *P. guttatus* were found by Caillouet et al. (1971) in Florida in greatest abundance in June, and in declining numbers to October, but there were no data for November to May. In contrast, at Jamaica, *P. guttatus* was taken in June, November and March, and Munro (1974) concluded that it is likely that spawning occurs in all months. In contrast, at the northern point of the Gulf of Mexico and Caribbean region (Bermuda), spawning of *P. guttatus* was found to be confined to a very short season in the present study, from May to August, possibly September, which is perhaps the briefest in its range.

The smallest ovigerous female *P. guttatus* captured by Caillouet et al. (1971) in Florida was 36 mm CL. From their data it would seem likely that the SAM for *P. guttatus* in Florida is in the range 36–50 mm CL. The cooler temperatures at Bermuda would be commensurate with a higher SAM. Morgan (1980) pointed out that SAM is generally greater for any species of lobster in cooler waters than for the same species in warmer waters.

The transect technique was used by Sutcliffe to study the reproductive biology of *P. argus* at Bermuda (Sutcliffe 1952, 1953). He found tentative evidence of an inward breeding migration of female *P. argus* lobsters from 'deeper waters of the shelf and of

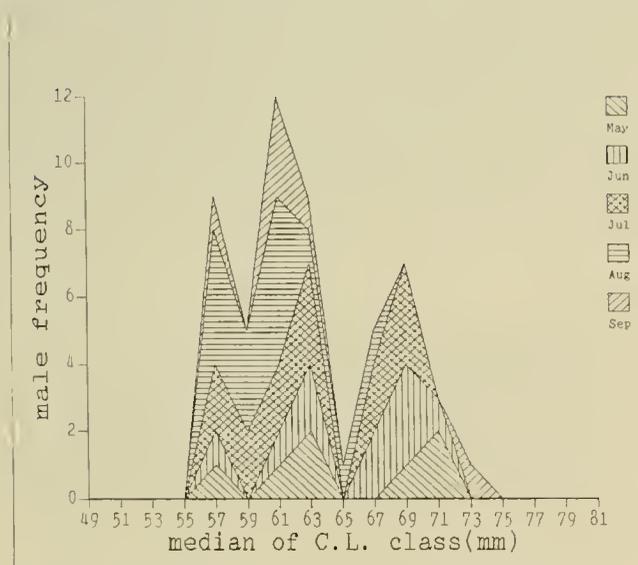


Figure 7. Chart showing monthly size frequency distributions of males caught by Transect Pot 8 (at Kitchen Tower). Transect Pot No. 8 was located close to the Kitchen Tower, and set in a sand-floored depression in the reef, close to overhanging coralgall cliffs at the reef-crest. It was the centre of the transect line, at the reef-crest. Size composition changed through the Summer to one of increasingly smaller size. Trappable *P. guttatus* of relatively small size are usually found seaward of the reef-crest on the reef-front terraces and slopes out to reefs of 43 metres depth (22 fathoms) (Evans 1988, 1989; Evans and Lockwood 1994).

the slopes' to the Nonsuch Area of the South Shore (chiefly less than 5 fathoms), and this apparently followed the first mating but preceded the hatching of eggs (Sutcliffe 1952). He also obtained evidence of an outward breeding migration of female and smaller male *P. argus* lobsters from the inshore areas, North Shore and North Lagoon, to the periphery of the Bermuda Platform, i.e., the outer reefs (Sutcliffe 1952, 1953). He found that larger males remained in the patch reefs of the lagoon, apparently as a result of their generally incompatible size in regard to mating; the sizes of spermatophores and recipient females are positively correlated (Sutcliffe 1953). In his brief study of *P. guttatus* at Bermuda, Sutcliffe (1953a) found no evidence of migration but it would seem that the underlying cause of the inward migration of *P. guttatus* in the more detailed present study was the same as found for *P. argus* by Sutcliffe (spawning and reproduction).

The disparity in the sex ratio of *P. guttatus* may be the result of (1) differential trap rates linked to the 50 mm CL mean retention length of the 4.1 mm hexagonal mesh traps, and the relatively small size of females compared to males (Evans 1988, 1989). Alternatively, it may be the result of (2) lower catchability of females, found by Evans and Lockwood (1994), or (3) lower abundance compared to males. The smaller size of females compared to males may result in greater natural mortality of early benthic juvenile females compared to males. The early benthic juvenile phase of palinurid lobsters is prone to high predation rates until a partial size refuge is attained (Smith and Herrnkind 1992). All three of these potential causes may contribute to the scarcity of trap-caught females, but the first listed is probably the chief cause of the disparity. Caillouet et al. (1971) found a sex ratio approaching unity, sampling by hand at Government Cut, Miami. Sutcliffe (1953a) found a sex ratio of male:female 10:1 at Bermuda, and Munro (1974) found a sex ratio of 77 males:37 females sampled in Jamaican waters. Munro (1974) pointed out that in both the latter cases, the mesh sizes of the traps probably precluded complete sampling of the female populations.

In the present study, *P. guttatus* was found only on the outer reefs, from the patch-reef zone of the lagoon to the edge of the island shelf, and in a depth range of 6–43 metres (Table 3) (Evans and Lockwood 1994), showing a differentiation of habitat in comparison with the Bermuda Spiny Lobster *P. argus*, which was

found in all parts of the island shelf (including the shallowest areas). This is in close agreement with the results of Munro (1974) at Jamaica who found that *P. guttatus* appeared to be confined to the relatively shallow water of <20 metres depth and to be most common in fairly exposed areas near the reef crest, though *P. guttatus* was found down to somewhat deeper depths at Bermuda, and it takes advantage of the ideal habitat afforded by the wide northeastern (18 metre depth) reef-terrace. This differentiation of habitat, shown by *P. guttatus* albeit with considerable overlap, is not clearly attributable to direct competition. However, related Palinurid species at Sri Lanka, East Africa and Northeastern Australia also show preferential depth, habitat and behaviour (De Bruin 1962, Kanciruk 1980). These preferences in the Indo-Pacific could have been initiated in order to avoid excessive inter-specific competition (Pollock 1992) and we can speculate this also for the differentiation observed on the Bermuda Platform and the island shelves of the Caribbean.

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## APPENDIX

*Calculation of the Intercepts***A. Results of Female *P. argus* LLI Intersect Analyses**

$$y = +0.650x - 0.242 \quad (1)$$

$$y = +0.521x + 10.851 \quad (2)$$

$$0 = +0.129x - 0.242 - 10.851 \quad \text{Eq. (1)-Eq. (2)}$$

$$+0.242 + 10.851 = 0.129x$$

$$11.093 = 0.129x$$

$$x = 11.093/0.129 = 86.0 \text{ mm CL}$$

**B. Results of Male *P. argus* LLI Intersect Analyses**

$$y = +1.038x - 36.405 \quad (3)$$

$$y = +0.672x - 0.739 \quad (4)$$

$$0 = 0.366x - 36.405 + 0.739 \quad \text{Eq. (3)-Eq. (4)}$$

$$36.405 - 0.739 = 0.366x$$

$$35.666 = 0.366x$$

$$x = 35.666/0.366$$

$$x = 97.4 \text{ mm CL}$$

**C. Results of Female *P. guttatus* LLI Intersect Analyses**

$$y = 1.104x - 19.383 \quad (5)$$

$$y = +0.426x + 20.565 \quad (6)$$

$$0 = +0.674x - 19.383 - 20.565 \quad \text{Eq. (5)-Eq. (6)}$$

$$19.383 + 20.565 = 0.674x$$

$$x = 39.948/0.674$$

$$x = 59.3 \text{ mm CL}$$

**D. Results of Male *P. guttatus* LLI Intersect Analyses**

$$y = 1.339x - 18.749 \quad (7)$$

$$y = +0.793x + 19.109 \quad (8)$$

$$0 = +0.546x - 18.749 - 19.109 \quad \text{Eq. (7)-Eq. (8)}$$

$$18.749 + 19.109 = 0.546x$$

$$x = 37.858/0.546 = 69.3 \text{ mm CL}$$



## RESETTLEMENT OF ICELAND SCALLOP (*CHLAMYS ISLANDICA*) SPAT ON DEAD HYDROIDS: RESPONSE TO CHEMICAL CUES FROM THE PROTEIN-CHITINOUS PERISARC AND ASSOCIATED MICROBIAL FILM\*

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**ABSTRACT** Laboratory experiments were carried out to determine whether the protein-chitinous perisarc of hydroids and/or associated biofilm (bacteria, microalgae and detritus) are involved in the association between the spat of the Iceland scallop *Chlamys islandica* (Mollusca: Bivalvia) and dead perisarc of the hydroid *Tubularia larynx* (Coelenterata: Hydrozoa). Results showed that post-larvae resettled in greater number on clean protein-chitinous perisarc rather than on perisarc fouled by biofilms. Single choice experiments also indicated that Iceland scallop spat resettled in greater number on the protein-chitinous perisarc of dead hydroids (60%) compared to other substrata (byssal threads of *Mytilus edulis*, surgical threads, strips of prawn exoskeleton and deproteinized strips of prawn exoskeleton). Results indicated that a significantly higher number of spat resettled both on deproteinized strips of prawn exoskeleton (composition close to pure polysaccharide) and byssal threads of *Mytilus* (high-molecular-weight collagenous protein) than on untreated prawn exoskeleton strips and surgical thread. This work suggests that high-molecular-weight proteinaceous component and/or polysaccharide, present in perisarc of dead hydroids, may be involved in resettlement of Iceland scallop spat on dead *T. larynx*.

**KEY WORDS:** *Chlamys islandica*, scallops, settlement, chemical cues, chitin

### INTRODUCTION

Many physical, biological and chemical factors are involved in the larval settlement process of marine benthic invertebrates. Physical factors such as water flow, substratum type, texture and heterogeneity, and light can be critical for the selection of settlement sites (Rittschof et al. 1984, Butman 1987, Bourget 1988, Le Tourneux and Bourget 1988, Harvey et al. 1995a,b). Chemical cues have also been identified as important larval settlement inducers in many marine invertebrate species (Burke 1983, Bonar et al. 1990, Pawlik 1990, Boudreau et al. 1993). Site-specific chemical cues have been intensively studied over the last decade, but few natural inducers of settlement have been isolated and characterized (Pawlik 1992). These inducers are usually: (1) peptides (Highsmith 1982, Rittschof 1990, Zimmer-Faust and Tamburri 1994) and/or free fatty acids (Pawlik 1986) associated with conspecifics or congeners, (2) hexane and ethanol extracts from brown and red algae (Kato et al. 1975, Yvin et al. 1985), (3) polysaccharides and glycoproteins from bacterial films (Hadfield 1986), and (4) polypeptides (Morse 1990) and sulphating polysaccharides (Morse 1991) associated with prey species.

Investigations on the chemical basis of the gregarious settlement of barnacles carried out during the last 50 years has resulted in the identification of a settlement factor called "arthropodin" (Gabbott and Larman 1987). This settlement factor is a high-molecular-weight proteinaceous component of arthropod cuticle, and was present in extracts of several barnacle species and other invertebrates including 2 species of sponges and the common crab *Carcinus maenas* (Crisp and Meadows 1962). More recently, Zimmer-Faust and Tamburri (1994) found that the waterborne chemical cue inducing settlement of planktonic oyster larvae

(*Crassostrea virginica*) was a low-molecular-weight peptide with arginine at the C-terminal. In scallop species, except for "jacarone", an algal extract isolated from the red algae *Delesseria sanguina* which induce larval metamorphosis in *Pecten maximus* (Chevolot et al. 1991), no natural larval settlement inducer has been identified yet.

Harvey et al. (1993) showed that in the Baie des Chaleurs (Gulf of St. Lawrence), newly settled Iceland scallop (*Chlamys islandica*) spat were mostly attached to the perisarc of dead hydroids (*Tubularia larynx*). Distributional data from field observations and results from laboratory dish assays, carried out to examine the ability of scallop post-larvae to select and resettle (ability to reattach by byssal threads on a new substratum) on various substrata, showed the existence of a strong relationship between post-larvae and dead hydroids (associative settlement, *sensu* Crisp 1974). It was therefore suggested that the patterns of distribution (large number of spat on dead *T. larynx*) observed in the field could result when spat redispersed to find more appropriate microhabitats after initial settlement on low-quality substratum. Redispersal would involve 4 phases: (1) dislodgement from the substratum, (2) bysso-pelagic transport and contact with a new substratum, (3) evaluation of habitat suitability, and (4) reattachment by byssal threads. Recent laboratory observations showed that spat of *P. maximus* and *Aequipecten (Chlamys) opercularis* were sometimes able to slow their sinking rate in a water column (2.3 m high) by producing a long fine byssal drifting thread (Beaumont and Barnes 1992). Furthermore, laboratory assays carried out by Harvey et al. (1993) showed that Iceland scallop spat: 1) can resettle when dislodged from their substratum, and 2) preferred to resettle on dead hydroids rather than on other substrata. They proposed that the recognition of dead hydroids by spat of Iceland scallop involved a chemotaxis behaviour or detection of chemical products (metabolites or extracts) diluted in the seawater surrounding the hydroids. Nevertheless, it remained unclear whether spat resettled preferentially on dead hydroids in response to chemical cues originating

\*Contribution to the program of GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec).

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from the perisarc, the protein-chitinous envelope secreted by the epidermis of the hydroid, or from the biofilm [bacteria, microalgae, and detritus (Hudon and Bourget 1981)] fouling the perisarc.

The present study is the first step of an ongoing program aimed at identifying natural settlement inducers involved in the settlement of scallop larvae. More specifically, this paper reports results of single-choice still water laboratory experiments designed to test the hypothesis that the biofilm fouling the perisarc of *T. larynx* plays a role in inducing settlement of Iceland scallop spat. This was carried out by conducting laboratory resettlement experiments with perisarc of dead hydroids untreated and treated with antibiotics. This paper also compared the resettlement patterns of Iceland scallop spat on different proteinous and chitinous substrata to evaluate the likelihood that settlement of Iceland scallop spat on dead hydroids is controlled by both proteins and/or chitin, which are the main constituents of dead perisarc of the hydroid *T. larynx*.

## METHODS

### General Experimental Procedure

The ability of Iceland scallop post-larvae to select and resettle on hydroids (treated and untreated with antibiotics) and on different chitinous and non-chitinous substrata was examined in 2 types of laboratory experiments carried out with *C. islandica* spat from the Baie des Chaleurs (Québec, Canada) in 1991, 1992 and 1993. Scallop spat used in resettlement assays were obtained from dead hydroids (*T. larynx*) following the technique previously described by Harvey et al. (1993). Spat had a shell length varying from 250 to 500  $\mu\text{m}$ . Care was taken to use individuals covering the entire size range for each assay. All experiments were performed in the dark at 8°C ( $\pm 1^\circ\text{C}$ ). Assays were carried out in 70  $\times$  50 mm crystallizing dishes (Corning no. 420630) filled with 100 mL of filtered seawater (Whatman, GF/C). Data were expressed as the % of the initial number of post-larvae that resettled by the end of the assay. Resettled individuals corresponded to those firmly attached to the substrata by byssal threads. This was verified by gently shaking the substrata on the bottom of the dish before counting resettled individuals. After each experiment, substrata were immediately frozen in filtered seawater and subsequently examined by scanning electron microscopy (SEM).

### Experiments with Antibiotics

This experiment was carried out with segments of dead *T. larynx* perisarc treated and untreated with antibiotics. Dead perisarc were characterized by the absence of their living content (i.e., coenosarc). Treated segments were obtained by placing dead perisarc in a mixture of equal amounts of penicillin G, streptomycin sulphate and tetracycline, at a final concentration of 100 ppm in sterile seawater (Johnson et al. 1991). Perisarc were exposed to antibiotics for a period of 24 hr. After treatment, the hydroids were washed in several baths of sterile seawater and gently brushed to remove antibiotics, lysed cells of bacteria, microalgae and detritus. Untreated hydroids (control) were kept in sterile seawater during the same period and were not washed before being used in experiments. Both treated and untreated hydroids were examined by SEM to evaluate the efficacy of this technique to eliminate and remove lysed cells of fouling bacteria and microalgae.

Twenty scallop spat were placed in crystallizing dishes containing 5 perisarc (3 cm long) of treated or untreated dead hy-

droids. The number of post-larvae resettled on each substrata were counted after a period of 12 hr. This experiment was initially done in 1991 and then repeated in 1992, to test reproducibility. In 1991 and 1992, assays were replicated 5 and 6 times, respectively.

### Experiments with Different Chitinous and Non-Chitinous Substrata

The hypothesis that both proteins and chitin play a role in inducing settlement of Iceland scallop spat on dead hydroids was tested by conducting laboratory resettlement experiments with different substrata. Five different types of substrata were used in these experiments: (1) dead perisarc (DP) without coenosarc and devoid of epibiota used as control, (2) byssal threads of *Mytilus edulis* (BT), (3) surgical threads (ST), (4) strips of prawn exoskeleton (SPE), (5) deproteinized SPE (D-SPE). DP and SPE have a protein-chitinous composition whereas D-SPE are mostly chitin, a polysaccharide (N-acetyl-D-glucosamine connected by  $\beta$  linkages) found in the exoskeletons of a large variety of marine invertebrates (Muzzarelli 1985). The rigid portion of BT used in the assays has a composition nearing pure collagen (Benedict and Waite 1986), a compound consisting of molecules containing 3 polypeptides,  $\alpha$ -chains, arranged in a triple helical conformation (Nimni and Harkness 1988). ST is a supramid white multifil (Serag-Wiessner Naila) composed of Polyamid-6 (or Nylon-6) which is produced by the polymerisation of caprolactam.

DP and BT were obtained each day of experimentation directly from the sea by SCUBA divers. SPE were obtained by cutting several 0.5 mm wide strips of prawn (*Penaeus sp.*) exoskeleton (cephalothoracic portion) bought from the fish market and kept frozen at  $-20^\circ\text{C}$  until used. Strips of prawn exoskeleton were deproteinized (D-SPE) with 2N NaOH for 2 hr at  $65^\circ\text{C}$  (No et al. 1989). After deproteinization, D-SPE were washed successively in running fresh and filtered seawater for about 30 min before being used in experiments.

Five groups of 15 freshly collected spat were respectively offered one of the five substrata for attachment. Each substrata was triplicated. All substratum trials (3  $\times$  5 dishes) were run at the same time. Dishes were randomly distributed on the same shelf in the refrigerator. Each substratum was 3 cm long and their number in a dish varied among types of substrata in order to have an approximately equal area for spat attachment (5 perisarc of dead hydroids, 10 byssal threads of *M. edulis*, 10 surgical threads, and 5 strips of treated and untreated prawn exoskeleton). The number of post-larvae which resettled on each substratum were counted after a period of 12 hr. This experiment was repeated three consecutive days using three different freshly collected batches of spat and renewed substrata. A total of 135 (3  $\times$  3  $\times$  15) spat was tested per type of substratum.

### Scanning Electron Microscopy (SEM)

The microtopography of the surface of each chitinous substratum used (DP and SPE), as well as bacteria, microalgae and detritus on the surfaces of both treated and untreated perisarc of dead hydroids, were examined by SEM. The samples, frozen immediately after experimentation, were thawed, rinsed with filtered seawater for 1 min, mounted on stubs, frozen at  $-20^\circ\text{C}$ , then freeze dried for 24 hr and coated with a gold-palladium film before examination.

### Data Analysis

Statistical analyses were carried out on arcsine-transformed data. This transformation increased homogeneity and normalized

the data expressed as percentages (Zar 1984). Given that variances were homogeneous and no major departure from normality were observed, unpaired *t* tests were used in experiments with antibiotics. On the other hand, since homogeneity of variances and normality were not respected, a non-parametric randomized block analysis of variance (Friedman's test) with multiple observations per cell (Conover 1980) was used in experiments with different chitinous and non-chitinous substrata. This test was followed by a non-parametric multiple range test among means. The null hypothesis for all experiments was that resettlement was the same on all substrata.

## RESULTS

Resettlement assays, carried out with hydroids treated and untreated with antibiotics, showed that a greater number of post-larvae resettled on clean chitinous perisarc than on perisarc fouled by a biofilm (epiphytic bacteria, microalgae and/or detritus) (Fig. 1). In 1991, the number of spat resettling on perisarc treated with antibiotics was significantly greater than on untreated perisarc ( $t = 2.44$ ,  $df = 8$ ,  $P = 0.04$ ). Resettlement was also greater, in 1992, on hydroids treated with antibiotics (mean  $\pm$  SE =  $36.67 \pm 6.66\%$ ) than on untreated hydroids (mean  $\pm$  SE =  $18.23 \pm 7.44\%$ ), although that year the difference was not significant ( $t = 1.83$ ,  $df = 10$ ,  $P = 0.09$ ).

Single choice experiments indicated that Iceland scallop spat resettled in significantly greater number on dead perisarc (DP) of the hydroid *T. larynx*, used as a control, rather than on other substrata used in these assays [byssal threads of *M. edulis* (BT), surgical threads (ST), strips of prawn exoskeleton (SPE) and deproteinized SPE (D-SPE)] ( $\chi^2 = 29.138$ ;  $P < 0.001$ ;  $n = 45$ ). Over 60% of spat resettled on DP compared to 44% for D-SPE, 31% for BT, 14% for SPE and 6% for ST (Fig. 2). Results also indicated that a significantly higher number of spat resettled on both deproteinized strips of prawn exoskeleton (D-SPE) and byssal threads (BT) than on untreated strips of prawn exoskeleton (SPE) and surgical threads (ST) (Fig. 2).

## DISCUSSION

Results from antibiotic experiments showed that untreated perisarc (Fig. 3a,b) were not as attractive as treated ones (Fig. 3c). Although this trend was the same for both years, discrepancies

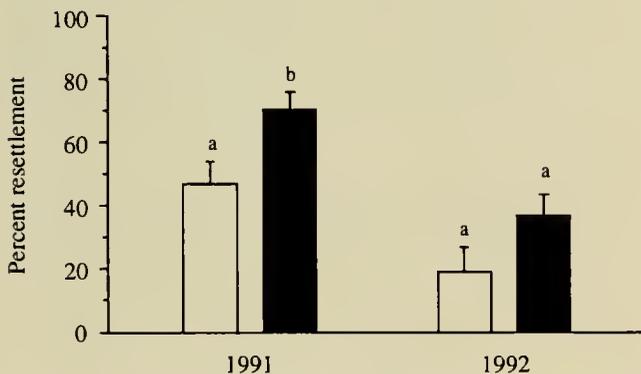


Figure 1. *Chlamys islandica*. Mean percent ( $\pm$ SE) spat resettlement on the perisarc of dead hydroids treated (black) and untreated (white) with antibiotics in both 1991 and 1992. Bars having dissimilar letters above them differ significantly from each other (Students *t*-tests: 1991,  $t = 2.44$ ,  $df = 8$ ,  $P = 0.04$ ; 1992,  $t = 1.83$ ,  $df = 10$ ,  $P = 0.09$ ).

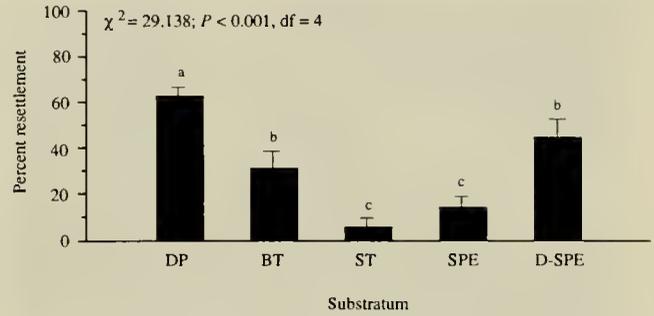
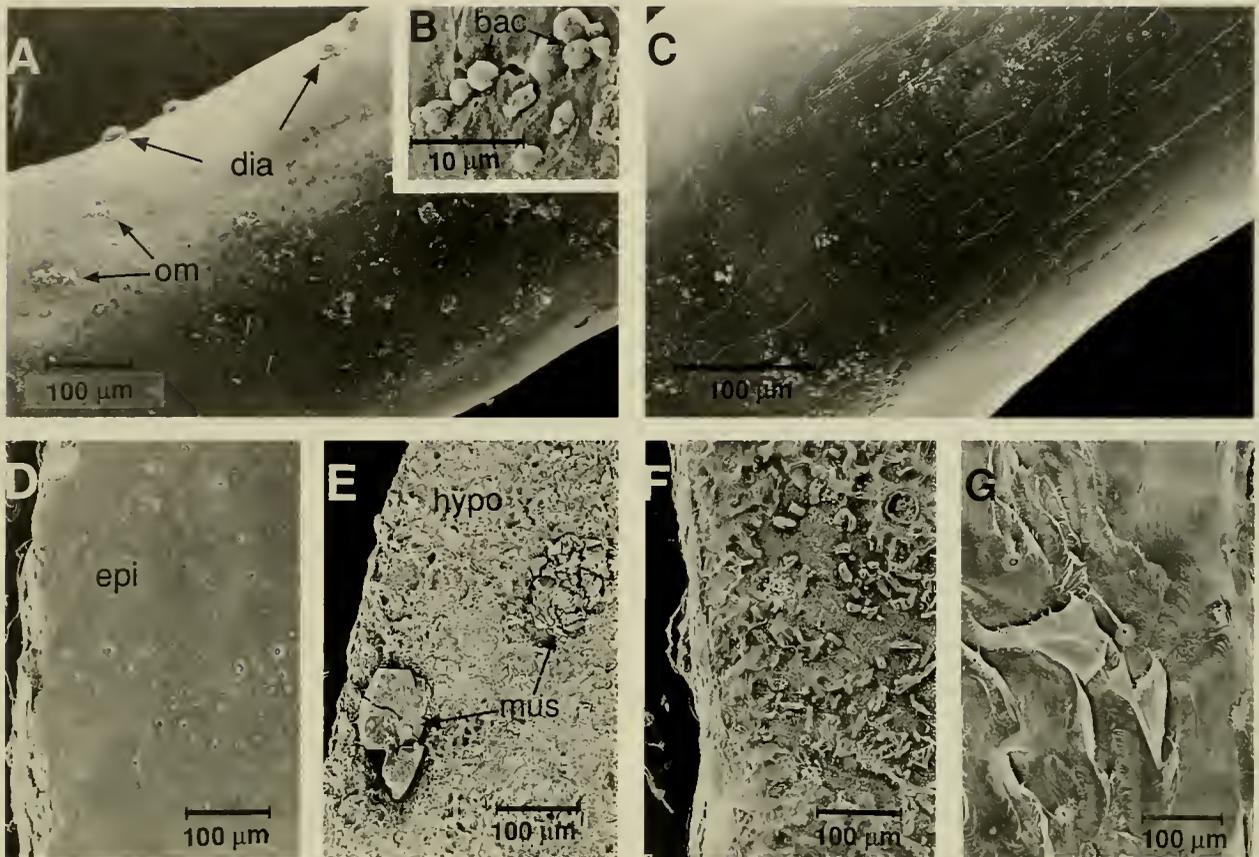


Figure 2. *Chlamys islandica*. Mean percent ( $\pm$ SE) spat resettlement on different substrata. Bars having dissimilar letters above them differ significantly from each other (Friedman's). DP, dead hydroids used as control; BT, byssal thread of *Mytilus edulis*; ST, surgical thread; SPE, strips of prawn exoskeleton (*Penaeus* sp.); D-SPE, deproteinized strips of prawn exoskeleton.

between untreated and treated perisarc were not significant in 1992. Moreover, the significant effect observed in 1991 was weak ( $P = 0.04$ ). Overall, it appears that the biofilm associated with the perisarc of dead hydroids (*T. larynx*) do not play an important role in the resettlement behaviour of *C. islandica* spat. Surface-colonizing marine bacteria have been shown to have both inhibitory (Maki et al. 1990, Holmström et al. 1992) as well as inductive (Bonar et al. 1990, Maki et al. 1990) effects on the settlement behaviour of some marine invertebrate species. An inhibitory effect was observed in barnacles (Maki et al. 1990), ascidians (Holmström et al. 1992), and bryozoans (Maki et al. 1989). Except in *Pecten maximus*, for which bacterial films were not shown to influence settlement (Tritar et al. 1992), both mixed biofilms (bacteria, microalgae and detritus) and specific bacterial films, developed on artificial (monofilament gill netting) and natural (cultch) substrata, were shown to increase settling intensity of all scallops species examined [e.g., *Chlamys hastata* (Hodgson and Bourne 1988), *Patinopecten yessoensis* (Foighil et al. 1990), *Argopecten irradians* (Xu et al. 1991) and *Placopecten magellanicus* (Parsons et al. 1993)]. There is no simple explanation as to why different scallop species show different attachment responses to biofilms. Larvae of some invertebrates species (e.g., barnacles) have been shown to change their attachment response to a bacterium when that bacterium was located on different surfaces (Maki et al. 1990). In other species (e.g., the crown-of-thorns starfish *Acanthaster planci*), bacteria must be associated with a particular natural substrata (the crustose alga *Lithothamnium pseudosorum*) to induce larval settlement (Johnson and Sutton 1994). The positive or negative larval response to a given biological cue (a diatom) may even depend on the scale of observation (see Bourget 1988, Le Tourneux and Bourget 1988). Hence, responses may depend on the species composition of the microflora and/or on the type of substrata supporting the biofilms. In this study, the lack of effect of the biofilm on the spat resettlement of *C. islandica* suggest that resettlement cues may originate from hydroid perisarc themselves.

Spat used in experiment with different chitinous and non-chitinous substrata showed 3 categories of responses in relation to the type of substrata offered. Firstly, they resettled in greater number on dead hydroid perisarc (DP), the substrata on which they were predominantly associated with in the field (Harvey et al. 1993). Secondly, they resettled in equal proportion on both byssal threads of *M. edulis* (BT) and deproteinized strips of prawn exo-



**Figure 3.** Electron microphotographs of substrata. Surface details of untreated (A and B) and treated (C) dead hydroids (*Tubularia larynx*). Diatoms (dia), bacteria (bac) and debris of organic matter (om) were readily observed on untreated (A and B) dead hydroids, though not abundant, compared to treated (C) ones. Antibiotic treatment and subsequent washing of perisarc appeared to be effective in removing debris of organic matter, lysed cells of bacteria and microalgae. Untreated external (D) and internal (E) surfaces of strips of prawn exoskeletons showing the waxy epicuticle (epi) layer on the external side and the hypodermis (hypo) layer on the internal side. Debris of muscles (mus) protruding from the hypodermis were also observed on the internal side. Treated external (F) and internal (G) surfaces of strips of prawn exoskeletons showing that the deproteinization of the prawn exoskeleton was effective in removing the waxy epicuticle on the external side and debris of muscles and the hypodermis on the internal side. Following the NaOH treatment, the internal side of the prawn exoskeleton (G) appeared to be composed of several superimposed thin layers of chitin.

skeleton (D-SPE). Thirdly, they resettled in lesser proportion on untreated strips of prawn exoskeleton (SPE) and on surgical threads (ST) than on all other substrata offered. Except for ST which was a polyamid and for SPE which was covered externally with a waxy epicuticle (a cement layer constituting the external surface of the cuticle) and presented fragments of hypodermis and muscles on the internal side (Fig. 3d,e), all other substrata used in the experiment were either pure protein (BT), pure chitin (D-SPE), or a combination of both (DP). Given that Iceland scallop spat responded more favourably to a combination of both protein and chitin (DP), and to a lesser extent to pure protein (BT) and pure chitin (D-SPE), we hypothesize that high-molecular-weight proteinaceous components and/or polysaccharides, present in DP, are involved in the resettlement of Iceland scallop spat on the dead *T. larynx*.

Both high-molecular-weight proteinaceous components and polysaccharides have been shown to induce settlement and metamorphosis in some marine benthic invertebrates species (Hadfield 1986). In addition to "arthropodine", large proteins present in the exoskeletons of arthropods (Gabbott and Larman 1987) which induce barnacle settlement, extracellular polysaccharides or glycoproteins attached to the bacterial wall of certain marine bacterial films (Kirchman et al. 1982, Hadfield 1986) and sulphating

polysaccharides in the wall of coralline algae (Morse 1991) have been shown to induce settlement in both the polychaete *Janua (Dexiospira) brasiliensis* and the coral *Agaricia humilis*. Furthermore, Maki et al. (1990) proposed that although bacterial polysaccharides have been suggested to be important cues in the settlement and metamorphosis of barnacle cypris larvae, they may also act as binding components for other settlement regulatory components. Although this is speculative, polysaccharides in hydroid chitin could act as binding components for the high-molecular-weight proteins.

The above experiments emphasize the relationship between Iceland scallop spat and the perisarc of dead hydroids. Assays were not aimed at providing a precise identification of a settlement inducer involved in scallop larval settlement on dead hydroids, but to direct future research towards the isolation of molecules inducing settlement and metamorphosis of larval scallops. Our results suggest that those molecules could be high-molecular-weight proteins and/or polysaccharides present in chitin.

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## HETEROZYGOSITY AND METABOLIC EFFICIENCY IN THE SCALLOP *EUVOLA ZICZAC* (LINNEAUS, 1758)

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**ABSTRACT** Possible correlations between enzyme heterozygosity and reproductive activity, metabolic enzyme activities, and growth rate were assessed for an eastern-Venezuelan population of the scallop *Euvola ziczac*. The reproductive output (gonadosomatic index), an indicator of the capacity for maintenance energy expenditure (ATPase) in adductor muscle and gills, and an indicator of growth rate (RNA/DNA ratio) in muscle were not correlated with the enzyme heterozygosity. A significant correlation was found between the degree of enzyme heterozygosity and the maximal activity of pyruvate kinase, octopine dehydrogenase, and NADP-dependent dehydrogenases in the adductor muscle. This suggests that genetic variability influences the enzyme of energy metabolism and may affect the locomotory activity and organismal production.

**KEY WORDS:** Heterozygosity, scallops, metabolism, growth

### INTRODUCTION

Several authors have found a relationship between heterozygosity and growth rate in bivalves (Zouros et al. 1986, Koehn and Gaffney 1984, Diehl et al. 1986, Gentili and Beaumont 1988, Alvarez et al. 1989, Scott and Koehn 1990). However this association is not universal, as has been demonstrated for both sessile and motile species (Beaumont et al. 1983, Foltz and Chatry 1986, Bricelj and Krause 1992, Slattery et al. 1993).

There is also evidence for relationships between higher heterozygosity and more efficient basal metabolism, suggesting a lower energy expenditure for maintenance leaving more ATP available for fuel growth and anabolic processes (Koehn and Shumway 1982, Gaffney et al. 1984). Furthermore, it has been suggested that organisms could use this surplus energy to increase fitness, depending on individual requirements (Garton 1984, Rodhouse et al. 1986, Volkaert and Zouros 1989). Accordingly, in sedentary molluscs the extra energy could be directed to growth in juveniles and to reproduction in adults; in motile organisms the extra energy could be used for improving locomotory capacity. Interestingly, Volkaert and Zouros (1989) showed a correlation between heterozygosity and octopine accumulation in the scallop *Placopecten magellanicus* following locomotory activity.

The foregoing observations suggest that genetic variability in molluscs might, under natural selection, act on the metabolic pathways involved in the energy metabolism associated with the processes of locomotion, maintenance, growth and reproduction. Consequently, the changing patterns of enzyme activity underlying such functional parameters may reveal adaptational options for organisms facing environmental change or stress. Reiterative investigations are required to identify physiological or behavioural features which have evolved in distinct genotypic groups within bivalve species.

This study examined the relationship between enzyme heterozygosity and adaptive traits, such as growth capacities of enzymes of energy metabolism and gonadal activity in the scallop *Euvola ziczac*. The RNA/DNA ratio and the glycolytic enzymes pyruvate kinase (PK) and octopine dehydrogenase (ODH) were determined as indicators of growth capacity and functional anaerobic potential in the adductor muscle, respectively. Similarly, the NADP-oxydoreductases, glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme ( $\zeta$ MEP): isocitrate dehydrogenase ( $\zeta$ IDHP), as well as total ATPase were assayed to evaluate the capacity for anabolic and the energy expenditure in the adductor muscle. Total gill ATPase activity was also measured.

Given the importance of the gills for ionic balance and the adductor muscle for the processes which underly the overall energy metabolism in bivalves it is reasonable to use this tissue to study the biochemical strategies involved in pectinid survival.

### MATERIALS AND METHODS

Specimens of *E. ziczac* were obtained in Chacopatica, Gulf of Cariaco, Venezuela (10°25'–10°35'N, 63°40'8"–64°13'W). Six samples of 30 individuals each, collected in different times between January and September 1992, were analyzed.

Adductor muscle, digestive gland, gills and gonad from each animal were dissected, weighed, immediately frozen with liquid nitrogen, and stored at –40°C for further analysis.

Reproductive development was followed using the gonadosomatic index (GI) expressed as wet gonad mass (WGM)/shell length (GI = WGM/length × 100). This method minimized tissue exposure before freezing. In a preliminary study this measure of sexual development was found to be equivalent to expression of gonad mass as a percentage of body mass.

Individual heterozygosity was determined by measuring six enzyme systems found to be polymorphic in this species by Coronado et al. (1991), namely leucyl amino peptidase (LAP: 3.4.11) and glutamate pyruvate transaminase (GPT: 2.6.1.2) in digestive

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gland; esterases (ES: 3.1.1.-), malate dehydrogenase (MDH: 1.1.1.37), phosphoglucosyltransferase (PGM: 5.4.2.2) and octopine dehydrogenase (ODH: 1.5.1.11) in adductor muscle. The tissues were minced and homogenized with cold distilled water, followed by centrifugation at  $3000 \times g$  for 10 min. The supernatants were used for 10% horizontal starch gel electrophoresis.

For the metabolic enzyme assays, 8 animals with different genetic variability ranging in a scale between zero and one were selected, from each group of 30 individuals previously characterized for their isozyme patterns.

To measure enzyme activity, in the adductor muscle, we used the following homogenization buffer (20% w/v) in 100 mM triethanolamine, 1 mM EDTA- $\text{Na}_2$ , 1 mM DDT, 0.1% Triton X-100 pH 7.5, with 100  $\mu\text{M}$  phenyl methyl sulfonic acid. For ATPase, Triton X-100 was not used and sucrose 250 mM was employed in the homogenizing medium. Homogenates were centrifuged at  $15000 \times g$  at  $0-4^\circ\text{C}$  for 20 min in a Sorvall centrifuge RC2-B. Supernatants were used for the activity determinations of PK, ODH, G6PDH,  $s_1$ IDHP, and  $s_1$ MEP systems. For ATPase, the homogenates of adductor muscle and gills were centrifuged at  $10,000 g$  at  $0-4^\circ\text{C}$  for 15 min; supernatants were centrifuged again at  $30000 g$  for 30 min. Final pellets were resuspended in 1 mL of homogenization buffer, and used as the enzyme source.

Maximal enzyme activities were monitored at 365 nm in optimal substrate and pH conditions at  $25^\circ\text{C}$ , following NADH or NADPH oxidation in a Bausch & Lomb Spectronic 20 spectrophotometer coupled to B & L 10" Strip Recorder. Reactions were initiated by the addition of substrate to the following incubation mixtures. Appropriate controls without substrate were run for each enzyme assay. All pH values were adjusted at  $25-27^\circ\text{C}$ .

**PK (2.7.1.40):** 10 mM  $\text{MgCl}_2$ ; 100 mM KCl; 0.16 mM NADH; 2 mM phosphoenolpyruvate; 4 mM ADP; 50 mM imidazole-HCl for pH adjustment at 7.0 and an excess of lactate dehydrogenase.

**ODH (1.5.1.11):** 8 mM pyruvate; 75 mM arginine; 2.26 mM NADH; 50 mM imidazole-HCl for pH adjustment at 7.0.

**G6PDH (1.1.1.49):** 1 mM EDTA; 1 mM DTT; 5 mM  $\text{MgSO}_4$ ; 2 mM glucose-6P; 0.05 mM NADP; 50 mM imidazole, HCl for pH adjustment at 7.5.

**$s_1$ IDHP (1.1.1.42):** 1 mM EDTA; 1 mM DTT; 5 mM  $\text{MnSO}_4$ ; 2 mM Isocitrate; 0.05 mM NADP; 50 mM imidazole, HCl for pH adjustment at 7.5.

**$s_1$ MEP (1.1.1.40):** 1 mM EDTA; 1 mM DTT; 5 mM  $\text{MnSO}_4$ ; 2 mM malate; 0.05 mM NADP; 50 mM imidazole, HCl for pH adjustment at 7.5.

**ATPase:** 5 mM  $\text{MgCl}_2$ ; 20 mM KCl; 100 mM NaCl; 0.5 mM phosphoenolpyruvate; 0.05 mM fructose 1,6-biphosphate (F1,6BP); 3 mM ATP; 0.05 mg/mL oligomycin; 0.2 mM NADH; 30 mM tris-HCl and coupling enzymes in excess (PK/LDH) at pH 7.5.

Enzyme activities were expressed as international units per dry mass (IU/gdm). One international unit was defined as the amount of activity which oxidizes one  $\mu\text{mol}$  of NADH or NADPH/min.

Muscle concentrations of RNA and DNA were determined by the fluorometric method of Karsten and Wolleberg (1977). Muscle samples were homogenized in cold phosphate buffer (PBS) (0.1 g/3 mL) with an ultra speed Brinkmann Polytron. After sedimenting debris by a low speed centrifugation at  $4^\circ\text{C}$ , aliquots of 200  $\mu\text{L}$  of the supernatants were used for quantification of RNA and DNA. The fluorometric determinations of nucleic acids were made at 365 nm excitation and 590 nm emission on a digital

fluorometral Sequoia-Turner model 450. Sigma Calf Thymus DNA type I and Sigma Yeast RNA type VI were used as standards.

To determine possible relationships among shell length, heterozygosity, gonadosomatic index and enzymatic activities a multiple correlation analysis was employed. The threshold significance probability was computed by Bonferroni procedure in which a nominal P value of 0.001 was treated as being equivalent to 0.05 in each single test. Differences in enzyme activities among the distinct genetic groups were tested by one-way analysis of variance.

## RESULTS

All specimens of *E. ziczac* were adults, with a mean length of  $7.73 \text{ cm} \pm 0.51 \text{ SD}$ , mean height of  $7.37 \text{ cm} \pm 0.46 \text{ SD}$  and mean width of  $2.62 \text{ cm} \pm 0.43 \text{ SD}$ .

The reproductive pattern showed 2 peaks of maximal gonadal activity, the first between February and March, and the second in August (Fig. 1). There was no correlation between GI and heterozygosity ( $r = 0.03$ ,  $p > 0.001$ ) (Table 1), although in organisms of the same size class (6.0–6.9; 7.0–7.9; 8.0–8.9 cm), the GI increased with the genetic variability (Fig. 2). A tendency to increase with size was also observed. There was a negative correlation between GI and G6PDH ( $r = -0.49$ ,  $p < 0.001$ ) and between GI and ATPases ( $r = -0.50$ ,  $p < 0.001$ ;  $r = -0.48$ ,  $p < 0.001$ ) (Table 1).

Significant positive correlations between heterozygosity and the activity of PK and ODH (Table 2) were observed ( $r = 0.90$ ,  $p < 0.001$ ;  $r = 0.48$ ,  $p < 0.001$ ). There was no correlation between ATPase and heterozygosity ( $r = -0.11$ ,  $p > 0.001$ ;  $r = -0.02$ ,  $p > 0.001$ ). PK and ODH, as well as  $s_1$ IDHP and  $s_1$ MEP were positively correlated ( $r = 0.48$ ,  $p < 0.001$ ;  $r = 0.73$ ,  $p < 0.001$ ) (Table 1).

One-way ANOVA ( $F_s = 85.027^{***}$ ) and analysis of means (Duncan) formed 4 heterozygosity groups (0.2; 0.4; 0.6; 0.8) for PK activity. For ODH ( $F_s = 5.651^{**}$ ) 3 groups were identified (0.2–0.6; 0.4–0.6; 0.4–0.8) and for G6PDH ( $F_s = 5.117^{**}$ ) 2 groups were observed which were characterized by low (0.2 to 0.6) and high heterozygosity (0.6–0.8) respectively.

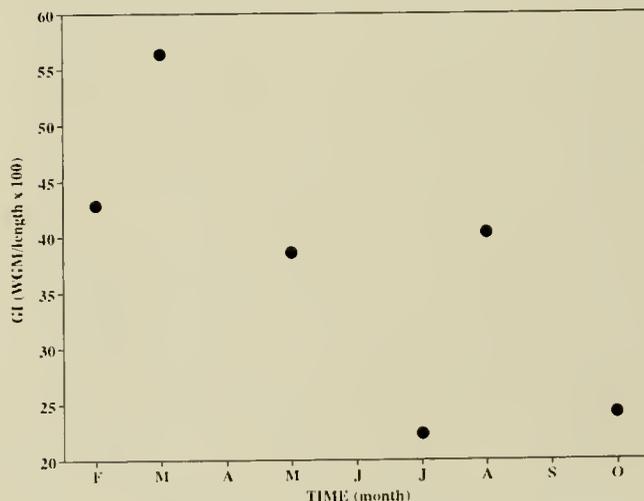


Figure 1. Gonadosomatic index patterns for different reproductive periods of *Euvola ziczac*. Each value represents the mean for 30 determinations.

TABLE 1.

Matrix of the overall correlation comparison among the different measured parameters.

	Length	Heter	GI	PK	ODH	G6PDH	sIDHP	sMEP	ATPaseM	ATPaseB	RNA/DNA
Length	-										
Heter	0.06 NS										
GI	0.49 *	0.03 NS	-								
PK	0.00 NS	0.90 *	0.10 NS	-							
ODH	-0.31 NS	0.48 *	-0.25 NS	0.48 *	-						
G6PDH	-0.28 NS	0.36 NS	-0.49 *	0.49 *	0.61 *	-					
sIDHP	-0.06 NS	0.32 NS	0.05 NS	0.19 NS	0.06 NS	-0.03 NS	-				
sMEP	-0.03 NS	0.30 NS	-0.03 NS	0.17 NS	0.11 NS	0.14 NS	0.73 *	-			
ATPaseM	-0.28 NS	-0.11 NS	-0.50 *	-0.08 NS	0.46 *	0.45 *	0.04 NS	-0.28 NS	-		
ATPaseB	0.20 NS	-0.02 NS	-0.48 *	0.03 NS	0.53 *	0.41 *	0.00 NS	0.01 NS	0.76 *	-	
RNA/DNA	-0.26 NS	-0.16 NS	-0.14 NS	-0.07 NS	-0.07 NS	0.14 NS	-0.10 NS	-0.21 NS	0.18 NS	0.12 NS	

ATPaseM = adductor muscle ATPase, ATPaseB = gill ATPase, numbers = r (correlation coefficient), \* = p < 0.001, NS = p > 0.001.

As ODH was also assayed by electrophoresis, the electrophoretic patterns obtained permitted determination of the ODH genetic variability and allowed the formation of ODH homozygote and heterozygote groups to evaluate genotypic influences on the ODH activity (Fig. 3).

The RNA/DNA ratio did not vary among the size groups, and was not affected by the degree of heterozygosity (Tables 1, 3).

DISCUSSION

This study suggests that genetic variability in *E. ziczac* is not associated with either reproductive development or the capacity for maintenance energy expenditure, but rather it is associated with enzymatic systems pertaining to energy metabolism whose

activities likely affect fitness. Seasonal enzyme fluctuations in major organs appear to be functional manifestations related to the integrative physiology of the annual reproductive cycle.

Although an increase in GI with heterozygosity for each size group of *E. ziczac* was found (Fig. 2), the general correlation between these 2 parameters was not significant. Furthermore, neither the morphometric data, nor the adductor growth rate (as measured by the RNA/DNA ratio) displayed an association with heterozygosity. Interestingly, the absence of a growth/heterozygosity relationship has been revealed among other wild pectinid populations; namely, *Pecten maximus* (Beaumont et al. 1985), *Placopecten magellanicus* (Foltz and Zouros 1984, Volckaert and Zouros 1989), *Argopecten irradians irradians* (Bricelj and Krause 1992).

The assessment of ATPase activity for the distinct genetic groups did not indicate any association between heterozygosity and basic energy expenditure in both gills and adductor muscle. Considering that the activity of ATPase might reflect maximal *in situ* oxygen demand in these tissues (adductor muscle and gills) in bivalves, characterized by a fairly high capacity for oxidative

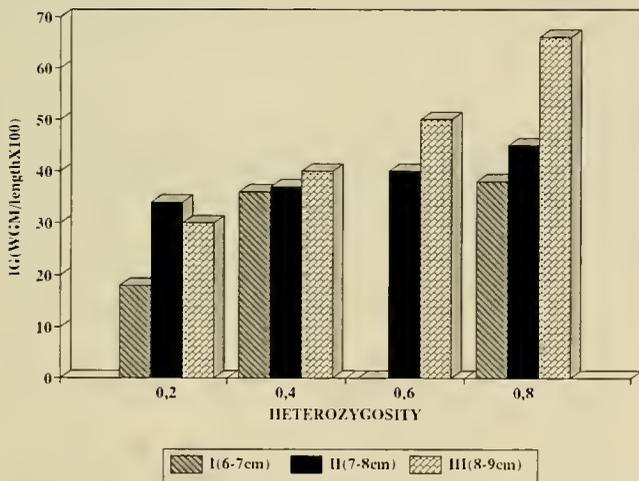


Figure 2. Gonadosomatic index for individuals of different size with different genetic variabilities.

TABLE 2.

Mean enzyme activities (U/g drywt).

Enzyme	Activity ± SD
PK	197.61 ± 58.24
ODH	21.78 ± 7.85
G6PDH	0.19 ± 0.13
sIDHP	7.32 ± 2.24
sMEP	4.35 ± 1.56
ATPaseM	1.27 ± 0.50
ATPaseB	1.44 ± 0.54

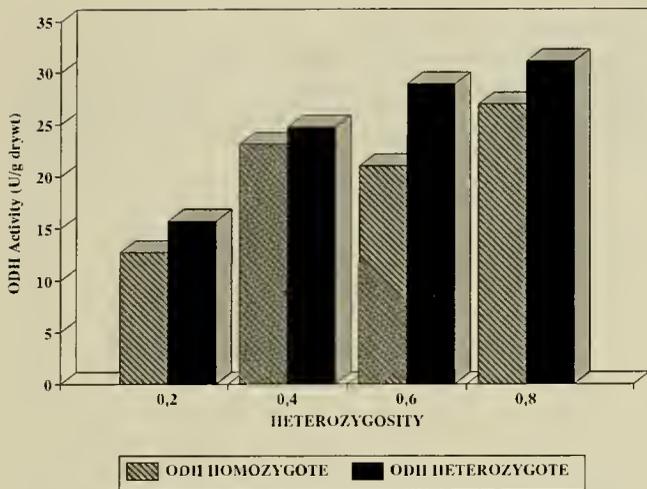


Figure 3. Octopine dehydrogenase activities in ODH homozygote and ODH heterozygote organisms with different individual heterozygosity.

phosphorylation (Guderley et al. 1994; Boada 1994); and that they might offer an important contribution to the overall energy cost of basal metabolism, it may be inferred that the use of fuels for maintenance is possibly a physiological trait unrelated to the degree of genetic variability. However, ATPase activity appears to be affected by reproductive condition; the inverse relationship between GI and ATPase signals that a decrease in the enzyme activity in the adductor muscle and the gills possibly leaves more energy available for reproduction.

In *Euvola*, the adductor muscle stores lipids which are mobilized during the period of gonadal maturation presumably in support of gametogenesis (Marcano 1993, Gómez 1991). G6PDH is a key enzyme of the pentose shunt, being a major generator of NADPH reductive equivalents for anabolic processes, particularly lipid synthesis. Consequently, the inverse relationship found between G6PDH and GI implies that G6PDH activity may be related to the reproductive processes that determine the redistribution of the energy flux through the gametogenic cycle. Thus, the enzyme function would be promoted during periods of low gonadal activity. Finally, for the case of the enzymes  $s_1$ IDHP and  $s_1$ MEP, their production of NADPH is associated with the metabolic use of carbon compounds from amino acids derived either from external or internal nutrient sources. It is worth noting that proteins are

TABLE 3.

Nucleic acid contents and the RNA/DNA ratio for the adductor muscle relative to the size of *Euvola ziczac* (Mean  $\pm$  SD).

Size	Class	RNA	DNA	RNA/DNA
I	(6–7 cm)	21.38 $\pm$ 5.01	4.31 $\pm$ 0.58	5.12 $\pm$ 0.11
II	(7–8 cm)	25.06 $\pm$ 5.72	5.49 $\pm$ 0.77	5.01 $\pm$ 0.95
III	(8–9 cm)	22.22 $\pm$ 5.42	7.15 $\pm$ 0.75	3.47 $\pm$ 0.17

important metabolic substrates for the adductor muscle in pectinids (Epp 1988, Gabbott, 1983).

The significant positive correlation between heterozygosity and maximal activities for PK and ODH might account for increased capacity for the generation of glycolytic ATP likely ensuring improved potential for locomotory activity and possibly metabolic substrate storage. Pyruvate kinase is a regulatory enzyme which catalyzes an essentially irreversible step in the glycolytic pathway, which in turn requires the activity of ODH to maintain the cytoplasmic NAD/NADH balance to accomplish its role in fueling the scallop muscle fibers during the escape response or catch closure (de Zwaan et al. 1980, Livingstone et al. 1981).

These underlying biochemical features lead to the assumption that heterozygosity confers functional adaptive strategies to optimize both the swimming escape response and reproductive development.

The enhanced capacity for muscle work associated with a higher degree of heterozygosity is consistent with higher enzyme activity for ODH loci of heterozygotes as compared with homozygotes. In addition, the individual genetic variability influences the enzyme by increasing the activity in ODH-homozygotes and ODH-heterozygotes with multilocus heterozygosity (Fig. 3). Borsa et al. (1992) pointed out that the multiloci heterozygosity effect is the consequence of an additive, direct effect at individual loci, with the loci involved in the energy production process displaying the strongest effects.

We conclude that most heterozygote individuals of *E. ziczac* are equipped with higher metabolic capacities for bouts of intense contractile activity involved in escape swimming. Similarly genetic variability might favor the development of raised anabolic potentials for growth. Finally, the changes in enzyme activities associated with the periodic variation of the gonadosomatic index reflect the integrative metabolic functions of the annual reproductive cycle.

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## SUMMER MORTALITY OF BLUE MUSSELS (*MYTILUS EDULIS* LINNEAUS, 1758) IN THE MAGDALEN ISLANDS (SOUTHERN GULF OF ST LAWRENCE, CANADA)

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**ABSTRACT** Off-bottom cultured mussels (*Mytilus edulis*, Linnaeus 1758) in the Magdalen Islands are frequently plagued by a severe summer mortality that can kill up to 80% of the individuals. A reciprocal transfer experiment using 4 local stocks was followed from June 1990 to November 1991. The stocks showed highly variable performances even though they came from neighbouring areas. A population resistant to the summer mortality was found as the survival rate of mussels originating from the Amherst Basin (AB) was 82%. Seed stocks used by the mussel industry from the Great Entry (GE) and the House Harbour (HH) lagoons had very low survival rates of 11% and 22% respectively. Single season results on survival are misleading and the study showed that utility of seed sources must be evaluated over the entire aquaculture production cycle to ensure accurate interpretation of results. For example, the 1-yr-old mussels from the open coast site at the Bay of Pleasant (BP) exhibited a survival rate of 92% and the 2-yr-old individuals a low 15%. The survival rate of the mussels (all stocks pooled) at the grow-out sites was comparable with 30-38%. Thus survival is influenced by a genetic (stock) rather than an environmental (site) component. Those GE individuals surviving summer mortality had high dry tissue masses and condition indices. The BP stock showed signs of stress (reduced growth rates, tissue masses and condition indices) during both years although its survival was very high during the first summer. The AB stock exhibited the best growth rates, tissue masses and condition indices in addition to its high resistance to summer mortality. We discuss our results in context of the various hypotheses proposed to explain summer mortality.

**KEY WORDS:** *Mytilus edulis*, summer mortality, stock, site, survival, growth, biomass

### INTRODUCTION

Summer mortality of cultured blue mussels (*Mytilus edulis*, Linnaeus 1758) is known from numerous studies from the Atlantic and the Pacific coasts of North America (Freeman and Dickie 1979, Incze et al. 1980, Skidmore and Chew 1985, Emmet et al. 1987, Bower 1989, Jamieson 1989, Mallet 1991, Sephton 1991). This has also been reported for the Pacific oyster, *Crassostrea gigas*, on the Pacific coast of North America (Perdue et al. 1981). The off-bottom cultured mussel industry in the Magdalen Islands in the southern Gulf of St Lawrence has been impeded regularly by summer mortality which can eliminate up to 80% of the individuals (Myrand 1991a, Myrand and Bergeron 1991). Until 1989, only the 2-yr-old mussels were lost but 1-yr-old individuals have also been decimated since then.

Several studies (Dickie et al. 1984, Mallet et al. 1987, Mallet and Carver 1989, Mallet et al. 1990, Sephton 1991, Fuentes et al. 1992) involving transfers of different stocks to various grow-out sites indicated that mussel survival is largely related to the origin of the stock (genetic component) whereas the rate of growth is related to the environmental conditions at the grow-out sites. Stocks with highly variable performances can be separated by relatively small geographical distances (Mallet et al. 1987, Sephton 1991) and Johannesson et al. (1990) reported that a substantial amount of genetic variation in some mussel allozyme loci can occur over very short distances, on the order of kilometers or less. Consequently, seed stocks resistant to summer mortality could possibly be found in the Magdalen Islands.

In the present study, a reciprocal transfer experiment was used at first to identify seed stocks resistant to summer mortality in the Magdalen Islands. Only local stocks were used because of the potential disease and parasite risks associated with bivalve transfers (Drinnan 1990, Gaffney and Allen 1992) and to provide the

most cost effective solution to the mussel growers. This study followed a preliminary experiment based on reciprocal transfer of 1-yr-old mussels in 1989 (Myrand 1990) that showed differential survival rates among the stocks from the Magdalen Islands.

Secondly, reciprocal transfers were used over a 2-yr cycle to get useful information on the summer mortality phenomenon and its causes. Different stocks kept at different grow-out sites were compared in order to distinguish between genetic and environment influences on survival rates and other related parameters (growth rates, biomass and condition indices). The comparison of the stocks' performance was used to characterize those with high and low survival. The comparison of the performances obtained at the different grow-out sites was used to investigate possible environmental causes of summer mortality. We were also interested in evaluating how interactions between stocks and grow-out sites could affect these parameters. Various hypotheses have been proposed over the years to explain summer mortality. They were examined thoroughly in the light of these results and a possible explanation for summer mortality of cultured mussels in the Magdalen Islands was proposed.

### METHODS

Mussel seed stock for the study was collected from 4 locations in the Magdalen Islands, an archipelago of about 70 km located in the southern Gulf of St. Lawrence (Fig. 1). At 3 locations, Great-Entry lagoon (GE), House Harbour lagoon (HH) and Amherst Basin (AB), seed mussels were obtained from collectors whereas those obtained from the Bay of Pleasant (BP) were taken from a navigation buoy anchor line. Seed were retrieved in early November 1989, approximately 3-4 months after initial settlement. The young mussels passed through a commercial declumper-grader machine that breaks up the clumps and eliminates the smallest

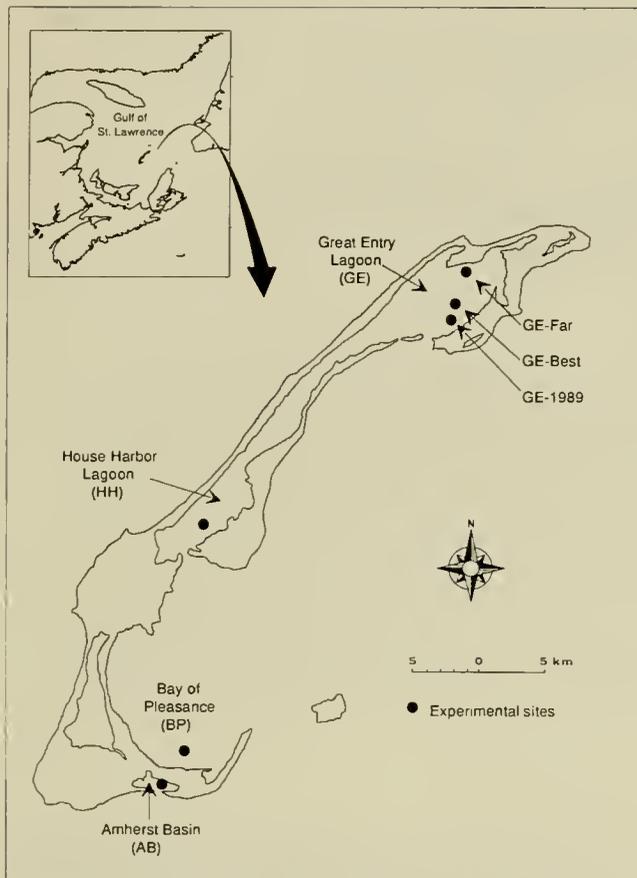


Figure 1. The Gulf of St Lawrence and the Magdalen Islands.

individuals. Seed from each stock was randomly separated in 12 groups and placed in numbered pearl-nets (4.5-mm mesh size), as used for commercial scallop culture. Density was approximately 350 mussels per net for the AB, GE and HH stocks whose mean shell length was comparable (15.2, 17.1 and 18.3 mm respectively). Density was approximately 625 spat per net for the BP stock whose mean length was much smaller (11.1 mm). The volume occupied by the mussels in the pearl nets varied from 150 mL (BP stock) to 375 mL (HH stock). Two pearl-nets from each stock were suspended from a long line at each of 6 experimental sites until next spring. The long lines were kept at least 2 m below the surface to avoid any ice damage during the winter.

The experimental sites (Fig. 1) were located at existing commercial culture operations in 3 well-sheltered lagoons: House Harbour lagoon (HH), Amherst Basin (AB), and Great Entry lagoon (GE). A growing site was also located in the Bay of Pleasant (BP) to have a full complement for a reciprocal transfer experiment. The AB, BP and HH experimental sites were the same as in the 1989 preliminary experiment (Myrand 1990). Spatial variability was assessed within the largest lagoon: the Great Entry lagoon. Three grow-out sites were dispersed in this lagoon which is characterized by a complex pattern of water circulation (Booth 1991). The GE-1989 site was the same as in the 1989 experiment, the GE-Best site was known as the best grow-out site according to mussel growers and the GE-Far site was located furthest from the entrance of the lagoon. Thus conclusions could be extended to the entire lagoons with no pseudoreplication (Hurlburt 1984). At each site a Ryan thermograph was placed near the experimental mussels from May to November to measure water temperature.

The experiment began in mid-June 1990 when all pearl-nets were recovered except those from the Bay of Pleasant which were lost. At each grow-out site, the mussels of each stock were pulled out of their pearl-nets, gently separated from each other and thoroughly mixed. Groups of 50 mussels were prepared to get adequate number of replicates with the same initial density. According to the number of individuals available, we obtained 4–6 replicates for each of the 20 stock  $\times$  site combinations. The groups of mussels were placed in numbered plastic cages (6,900 mL volume) with larger mesh size (9 mm) than in the pearl-nets in order to reduce fouling problems. Mussels  $<20$  mm were discarded because we previously observed that they could slip through the mesh.

A total of 22 to 24 cages were prepared for each grow-out site. They were randomly arranged in groups of three along a vertical axis so that the distance between the upper and the lower cages never exceeded 1 m. The groups of 3 cages were distributed randomly on a long line ( $\sim 1$  m from each other) and hung at a depth of 1 to 3 m from the surface to minimize turbulence. Depth was likely to have little effect because the waters of these shallow lagoons (maximum depth of 7 m) are very well-mixed (Booth 1994) and various authors reported that environmental conditions such as temperature, salinity, dissolved oxygen, seston and chl *a* are homogeneous through the water column (Auclair 1974, Mayzaud et al. 1989, Mayzaud and Souchu 1991, Myrand 1991b).

To minimize fouling, cages were changed at approximately monthly intervals until mid-November 1990. On each occasion, mussels were measured and dead ones removed. These samplings provided information on mean shell growth and on cumulative survival rate per cage ( $N_i \cdot 100/N_j$  June 90). We calculated the "monthly" survival rate for each successive *i* and *j* samplings as ( $N_j \cdot 100/N_i$ ) and the "monthly" growth rate as (mean length *j* – mean length *i*)/days between *i* and *j*. The "seasonal" survival and growth rates obtained from June to November were calculated on the same basis. Half of the cages from the each stock  $\times$  site combination were randomly selected during the November sampling and brought back to the laboratory for destructive measurements on the mussels. The remaining cages were left to overwinter under the ice.

In the laboratory, mussels from each stock  $\times$  site combination were mixed and 35 individuals were randomly selected and frozen. Later we measured their shell length before determining the dry mass of their tissues (65–70°C for at least 72 hr) and the damp-dried mass of the shells after removal of excess water with paper towelling. Shells were not oven-dried in order to minimize handlings associated with this kind of procedure. However, the damp-dried shell mass was converted to dry mass with the following formula:

$$\text{Shell dry mass (g)} = 0.978 \text{ Shell damp-dried mass (g)} - 0.010 \quad (r = 0.99, N = 109)$$

A condition index defined as the tissue dry mass:shell dry mass ratio (Mann 1978, Lucas and Beninger 1985, Aldrich and Crowley 1986, Davenport and Chen 1987, Rainer and Mann 1992) was calculated for each combination.

In 1991, the remaining cages were examined at approximately monthly intervals from early June to early November. Then they were brought back to the laboratory for measurements. All individuals were used when there were less than 35 mussels for the determination of the dry masses because of the cumulative effects of the 1990 and 1991 summer mortalities. In addition to the pre-

vious computations, we calculated the cumulative survival rate for the 2-yr-old mussels in 1991 as  $(N_i \cdot 100/N \text{ June } 91)$ . The overall 1990–1991 growth rate was estimated as  $(\text{mean length November } 91 - \text{mean length June } 90)/\text{days between June } 90 \text{ and November } 91$ .

All statistical analyses were done using SAS package (1982), version 6.03. The shapes of the cumulative survival curves were compared using the Lifetest procedure based on Log-Rank statistics. The procedure considers the individuals retrieved in November 1990 and those still alive in November 1991 as censored data. The survival rates were analyzed using 2-way factorial ANOVAs (stock  $\times$  site) applied to the angular transformed data (Sokal and Rohlf 1981). The growth rates were compared with factorial ANCOVAs using the mean length of the mussels at the beginning of the period under consideration as the covariable. Mass measurements and condition indices were compared using factorial ANCOVAs with individual shell length as the covariable. As stated by Packard and Boardman (1988), condition indices had to be adjusted using ANCOVA because they were not independent of the shell length ( $r = 0.36$ ,  $p < 0.0001$ ,  $N = 1170$ ). The multiple comparisons were performed on the adjusted means (unbalanced design) because of the uneven number of cages for the various combinations. Bonferroni's correction was always used to adjust the statistical threshold within the multiple comparisons in order to keep an overall acceptance threshold  $\alpha = 0.05$  (Miller 1981). The relative distribution of the variance for the annual survival rates was assessed with the VARCOMP procedure.

## RESULTS

### Survival

The shapes of the 20 survival curves analyzed with the Lifetest procedure varied significantly ( $\chi^2 = 2013.6$ ,  $DF = 19$ ,  $p < 0.0001$ ). Only the curves of the 4 stocks at each site were examined and then the performance of each stock at the 5 sites, since there were no simple means to consider each possible stock  $\times$  site interactions (Fig. 2; only the survival curves of the 4 stocks at each of the sites are presented). The apparent increases in survival observed in some cases between November 1990 and June 1991 (for example, from 17% to 27% for the GE stock at HH lagoon) merely reflected the random retrieval of half the cages in November. At any given site, the curves of the 4 stocks were statistically different ( $p < 0.05$ ) from each other. On the other hand, survival curves of a given stock were quite similar at all sites. For example, survival curves for any given stock were statistically identical at the sites HH, GE-Best and GE-Far.

Analysis of the "monthly" survival rate showed that the overall mean survival was always over 92% except for the period extending from July to September in both years (Fig. 3). Stock  $\times$  site interactions or difference in survival at the various sites during these low-mortality periods were not detected. The survival of the stocks only differed ( $p = 0.024$ ) during the very first period, mid June–late July 1990, when it was lower for the BP stock (90%) compared to the AB stock (99%); the other stocks showing intermediate survival values that were similar to these two. This lower performance of the BP stock could be mostly related to the loss of small individuals through the mesh of cages even though precautions were taken by using only individuals  $\geq 20$  mm. Considering the overall high survival, we focused on the July to September periods when the summer mortality occurred.

In 1990, the largest losses among the 1-yr-old individuals took

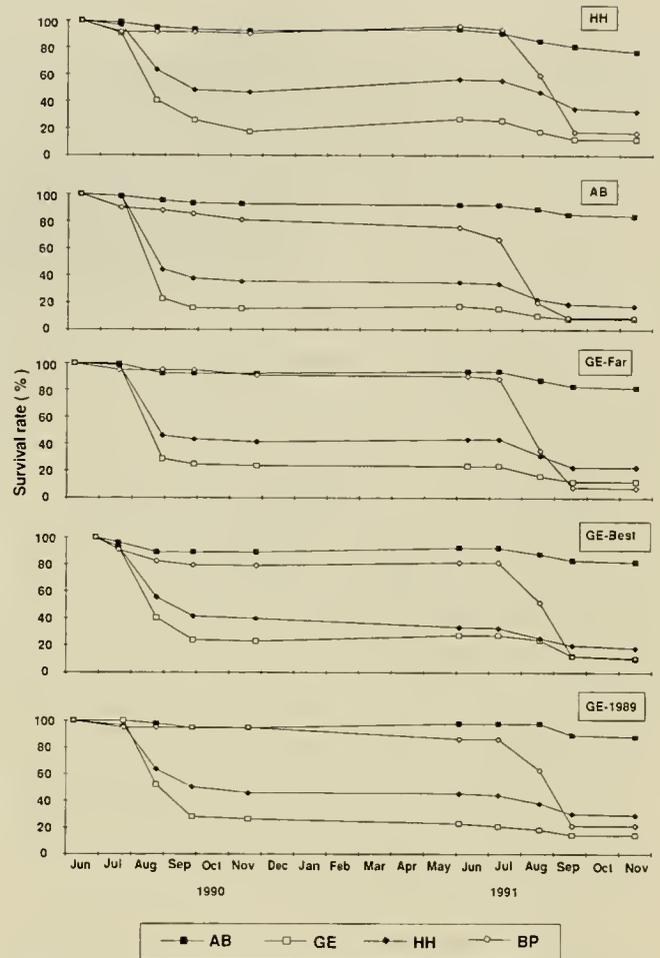


Figure 2. Changes in the survival rate of the 4 seed stocks grown at each of the 5 experimental sites from June 1990 to November 1991.

place during the late July–late August period as the overall mean survival rate was 69% (Fig. 3). Over the 2 years, there were never any significant stock  $\times$  site interaction except for this period ( $p = 0.029$ ). However, no specific differences were identified because the Bonferroni's correction lowered the acceptance threshold of each comparison to  $\alpha = 0.005$ . There was a general pattern for the stocks' survival at all sites:  $GE = HH < AB = BP$ . The mean survival during the late August–late September 1990 period was 85%. The pattern was slightly different from the previous period since the survival rate of the GE stock became significantly lower than the HH stock:  $GE < HH < AB = BP$ . Once again the survival was very similar at all sites. For example, no significant differences at 4 out of the 5 sites.

In 1991, the mean survival of the 2-yr-old mussels was 76% during the early July–early August period with a new pattern:  $BP < GE = HH < AB$ . This time the survival of the BP mussels was only 54% compared to 96% for the AB mussels. The survival was comparable at all sites since it was statistically identical at 4 out of the 5 sites, the fifth being comparable to two of them. The lowest mean survival rate ever recorded over the 2 years was 66% and was observed during the early August–early September period in 1991. The survival rate was again significantly lower for the BP mussels with 32% although the relation between the other stocks was not clearly defined with values ranging from 64% to 88%. The

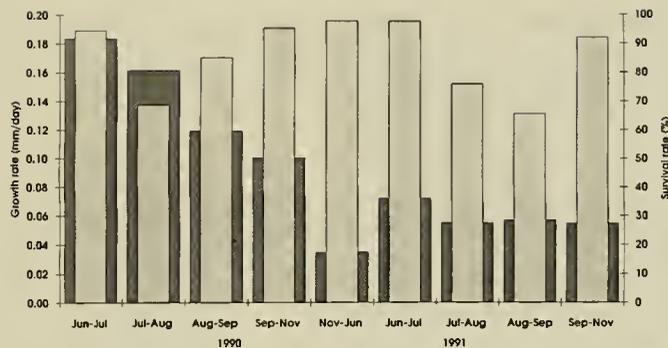


Figure 3. Mean values (all stocks and sites pooled) of the "monthly" growth rate (■) and survival rate (□) from June 1990 to November 1991.

survival at all sites was statistically identical ( $p = 0.31$ ) during this period.

The cumulative survival rates computed in November 1990 and 1991 are presented in Table 1. There were no stock  $\times$  site interactions ( $p = 0.32$  for 1990,  $p = 0.92$  for 1991,  $p = 0.64$  for 1990–1991). The cumulative survival for the AB stock was the highest for both years with 92% for 1-yr-old and 88% for 2-yr-old mussels. Over 2 years, 82% of these mussels survived. The BP stock had very different features in 1990 and 1991: one of the highest survival rates for the 1-yr-old mussels (87%) but the worst for the 2-yr-old individuals (15%). That resulted in the worst survival along with the GE stock after 2 years: 13% and 11% respectively. The GE stock had the lowest survival during the first year (23%) but a medium-range value for the 2-yr-old individuals. Finally, the HH mussels showed intermediate survival values for both years resulting in an overall survival of 22%.

The cumulative survival rate was quite similar at all GE sites in 1990 and statistically identical in 1991 (Table 1). Consequently, there were no significant differences in cumulative survival at the 3 sites located in the GE lagoon after 2 years. Still, cumulative survival was very similar at all the grow-out sites with values ranging from 30% to 38% after 2 years.

The mean cumulative survival rate was 60% for the 1-yr-old mussels in November 1990 and 52% for the 2-yr-old individuals in November 1991. Only 32% of all the mussels put in cages at the

beginning of the experiment were still alive in November 1991. The stock factor alone explained 87.1% and 82.2% of the cumulative survival variance in 1990 and in 1991, and 92.3% of the variance over the 2-year period. The site factor explained only 2.1% and 1.9% of this variance in 1990 and 1991 with an overall value of 1.6%.

#### Growth

The growth curves obtained from the mean shell length increase of the 4 stocks at each growing site are presented in Figure 4. Growth in terms of absolute length was generally comparable. The difference between the extreme mean length values observed at the beginning of the experiment lessened with time. In July 1990, the mean length per cage varied from 22.2 to 30.4 mm giving a difference of 37%. It decreased to 17% (45.1 to 52.8 mm) in November 1990 and to 15% (59.0 to 68.1 mm) in November 1991.

The "monthly" growth rate was compared for the successive sampling periods between June and November of both years (Fig. 3). In 1990, the mean growth of the 1-yr-old mussels had declined steadily over the experimental period from  $0.186 \text{ mm} \cdot \text{day}^{-1}$  during the mid June–mid July period to  $0.100 \text{ mm} \cdot \text{day}^{-1}$  during the late September–mid November period. In 1991, the highest mean rate was registered during the early June–early July period ( $0.072 \text{ mm} \cdot \text{day}^{-1}$ ) and was comparable afterwards with values between  $0.055$  and  $0.057 \text{ mm} \cdot \text{day}^{-1}$ .

The "monthly" growth rate of the different stocks was always identical except for 2 periods in 1990: late July–late August ( $p < 0.0001$ ) and late August–late September ( $p = 0.0004$ ). During the first period, it was highest for the BP stock ( $0.224 \text{ mm} \cdot \text{day}^{-1}$ ) followed by the AB stock ( $0.172 \text{ mm} \cdot \text{day}^{-1}$ ). The HH and GE stocks had the lowest rates ( $0.134$  and  $0.125 \text{ mm} \cdot \text{day}^{-1}$  respectively). During the late August–late September period, there was a significant interaction ( $p = 0.004$ ) but the growth rate was identical for all stocks at 4 out of the 5 sites. Globally, the "monthly" growth rate was comparable at all sites in 1990 and 1991. For example, it was always identical at 3 sites, and most of the time at 4 sites, for any given stock.

The "seasonal" growth rates and the 1990–1991 overall growth rates are presented in Table 2. There were no significant stock  $\times$  site interactions for any of these rates ( $p = 0.12$  for 1990,  $p = 0.10$  for 1991,  $p = 0.11$  for 1990–1991). The mean growth rate obtained in 1990 was  $0.139 \text{ mm} \cdot \text{day}^{-1}$ . More specifically, it was higher for the AB stock ( $0.157 \text{ mm} \cdot \text{day}^{-1}$ ) than for the

TABLE 1.

Cumulative survival rate per cage for the different stocks and the different sites (Mean  $\pm$  SE).

Factor	June to November 1990	June to November 1991	June 1990 to November 1991
<b>Stocks</b>			
AB	92.4 $\pm$ 1.6 <sup>a</sup>	87.7 $\pm$ 3.3 <sup>a</sup>	82.1 $\pm$ 1.7 <sup>a</sup>
BP	87.3 $\pm$ 1.7 <sup>a</sup>	15.1 $\pm$ 3.7 <sup>c</sup>	13.2 $\pm$ 1.9 <sup>c</sup>
HH	41.8 $\pm$ 1.5 <sup>b</sup>	54.7 $\pm$ 3.2 <sup>b</sup>	21.7 $\pm$ 1.6 <sup>b</sup>
GE	22.8 $\pm$ 1.5 <sup>c</sup>	47.4 $\pm$ 3.2 <sup>b</sup>	11.1 $\pm$ 1.6 <sup>c</sup>
<b>Sites</b>			
AB	56.6 $\pm$ 2.1 <sup>b</sup>	49.8 $\pm$ 4.4 <sup>a</sup>	29.7 $\pm$ 2.2 <sup>b</sup>
HH	63.9 $\pm$ 1.7 <sup>a</sup>	49.6 $\pm$ 3.7 <sup>a</sup>	31.6 $\pm$ 1.9 <sup>ab</sup>
GE-1989	64.8 $\pm$ 1.6 <sup>a</sup>	60.0 $\pm$ 3.5 <sup>a</sup>	38.1 $\pm$ 1.8 <sup>a</sup>
GE-Best	57.9 $\pm$ 1.7 <sup>b</sup>	47.9 $\pm$ 3.5 <sup>a</sup>	30.0 $\pm$ 1.8 <sup>ab</sup>
GE-Far	62.2 $\pm$ 1.6 <sup>ab</sup>	48.8 $\pm$ 3.5 <sup>a</sup>	30.8 $\pm$ 1.8 <sup>ab</sup>

The values followed by different letters in a given column are statistically different at an overall  $\alpha = 0.05$ .

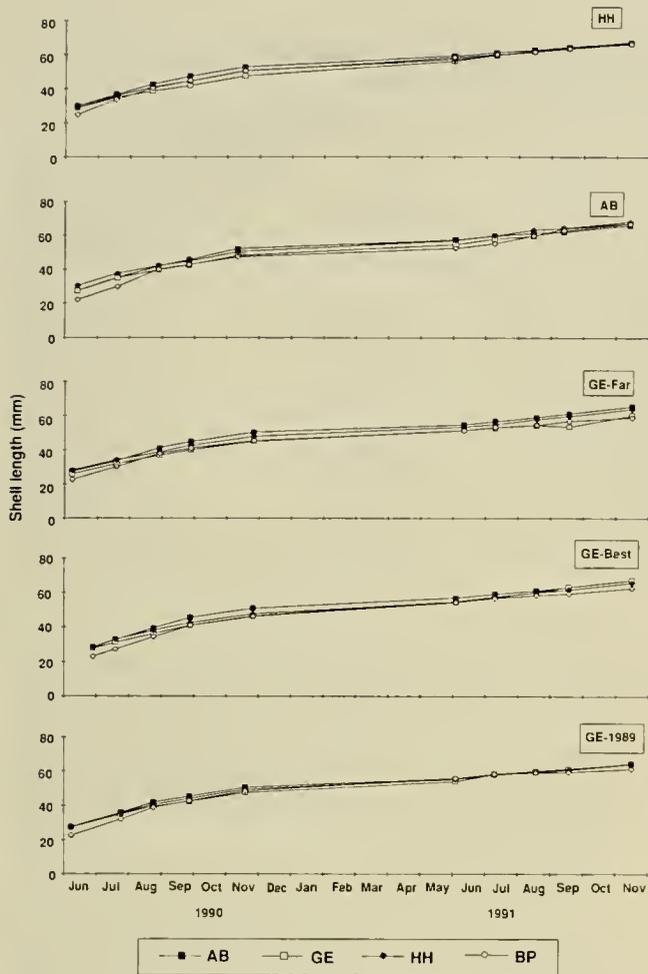


Figure 4. Changes in the mean shell length per cage of the 4 seed stocks grown at each of the 5 experimental sites from June 1990 to November 1991.

others. Growth rate was identical for the mussels kept at the AB and HH sites and significantly slower at the 3 GE sites. In 1991 the mean growth rate was  $0.060 \text{ mm} \cdot \text{day}^{-1}$ . It was similar for all the stocks except for the BP stock which was significantly slower with  $0.049 \text{ mm} \cdot \text{day}^{-1}$ . The best sites were AB, HH and GE-Best. The mean growth rate registered during the entire study was  $0.074 \text{ mm} \cdot \text{day}^{-1}$ . There were no differences between the stocks ( $p = 0.54$ ) after 2 seasons. The AB, HH and GE-Best sites were the best grow-out sites.

There was a positive partial correlation (eliminating the effect of the initial mussel length in June 1990) between the seasonal growth rate in 1990 and the cumulative survival in November of that year ( $r = 0.53$ ,  $n = 112$ ,  $p < 0.0001$ ). Data examined on a "monthly" basis showed that the most important relation between the 2 variables ( $r = 0.81$ ) was observed during the late July–late August period; a period of rapid growth and of low survival for some stocks. The relation (partial correlation without the effect of the mussel length in June 1991) was not so clear in 1991 ( $r = 0.27$ ,  $n = 57$ ,  $p = 0.041$ ). There were no significant correlations between the "monthly" growth rates and survival rates during the 2 periods plagued with summer mortality: early July–early August period and early August–early September period. Growth rates were low for all stocks during these periods (Fig. 3).

#### Tissue Masses and Condition Indices

Comparisons of the shell length–dry tissue mass relationships obtained in November 1990 were not possible because the slopes were not parallel ( $p < 0.05$ ), e.g., the relations among the treatments changed with the shell length under consideration. The SAS procedure compared the tissue masses adjusted to the mean shell length of the mussels used (50.0 mm in this case) but the conclusions should be restricted to this particular mean shell length. There was a significant stock  $\times$  site interaction ( $p < 0.0001$ ). In 1990, the BP mussels had usually a lower mass of dry tissue (0.63–0.76 g depending on the site) than the other stocks (Table 3). The GE stock had the highest tissue mass at all grow-out sites with values ranging from 0.68 to 0.94 g. The highest tissue dry mass was always produced by the mussels kept at the AB grow-out site. Tissue mass was usually similar at the 4 other sites (Table 3).

In 1991, the slopes of the shell length–dry tissue mass relationships were parallel ( $p = 0.08$ ) and the conclusions from the masses adjusted to the mean shell length (65.6 mm) could be extended over the full range of the shell lengths used: 44–79 mm. The stock  $\times$  site interaction was significant ( $p = 0.038$ ). The tissue mass was fairly similar for all stocks but when it differed, it was lower for the BP mussels (Table 3). The highest tissue masses for all stocks came primarily from the AB grow-out site (2.12–2.35 g) again.

The overall condition index was 0.195 in November 1990 and 0.219 in November 1991. Both years, the slopes of the stock  $\times$  site interactions were parallel ( $p = 0.18$  for 1990 and  $p = 0.26$  for 1991). They were compared and significant interactions were observed ( $p < 0.0001$  in 1990 and  $p = 0.04$  in 1991). In 1990, the BP mussels usually had a lower condition index (0.159–0.205) than the other stocks (Table 3). On the other hand, the GE stock had the highest index in most areas with values ranging from 0.188 to 0.234. The lowest condition indices were observed at one of the grow-out sites from the GE lagoon (GE-Best) whereas it was usually comparable at the other sites. In 1991, the AB stock had always the highest index (0.225–0.272) and again the BP stock the lowest (0.179–0.236). It was very similar at all sites since it was identical for at least 4 out of the 5 sites for all the stocks. However, the highest index always came from the mussels grown at the GE-1989 site with values from 0.194 to 0.272 according to the stock.

TABLE 2.

Adjusted growth rate per cage ( $\text{mm} \cdot \text{day}^{-1}$ ) for the different stocks and the different sites (Mean  $\pm$  SE).

Factor	June to November 1990	June to November 1991	June 1990 to November 1991
<b>Stocks</b>			
AB	$0.157 \pm 0.002^a$	$0.066 \pm 0.003^a$	$0.077 \pm 0.001^a$
BP	$0.133 \pm 0.005^{bc}$	$0.049 \pm 0.004^b$	$0.070 \pm 0.004^a$
HH	$0.142 \pm 0.003^b$	$0.067 \pm 0.003^a$	$0.077 \pm 0.002^a$
GE	$0.129 \pm 0.002^c$	$0.060 \pm 0.003^a$	$0.074 \pm 0.001^a$
<b>Sites</b>			
AB	$0.152 \pm 0.002^a$	$0.072 \pm 0.004^a$	$0.078 \pm 0.001^a$
HH	$0.150 \pm 0.002^a$	$0.070 \pm 0.004^a$	$0.078 \pm 0.002^a$
GE-1989	$0.137 \pm 0.002^b$	$0.051 \pm 0.003^b$	$0.070 \pm 0.001^b$
GE-Best	$0.141 \pm 0.002^b$	$0.064 \pm 0.003^a$	$0.078 \pm 0.001^a$
GE-Far	$0.121 \pm 0.002^c$	$0.046 \pm 0.004^b$	$0.068 \pm 0.001^b$

The values followed by different letters in a given column are statistically different at an overall  $\alpha = 0.05$ .

TABLE 3.

Adjusted masses and condition indices (dry tissue mass:shell mass ratio) calculated for a mussel of standard shell length (50 mm and 65.6 mm) from the different stocks and the different sites (mean  $\pm$  SE).

Factor	November 1990 (50.0 mm)		November 1991 (65.6 mm)	
	Dry Tissue (g)	Condition Index	Dry Tissue (g)	Condition Index
Stocks				
AB	0.77 $\pm$ 0.01	0.200 $\pm$ 0.003	2.06 $\pm$ 0.03	0.239 $\pm$ 0.004
BP	0.68 $\pm$ 0.01	0.183 $\pm$ 0.003	1.83 $\pm$ 0.06	0.197 $\pm$ 0.008
HH	0.79 $\pm$ 0.01	0.191 $\pm$ 0.003	2.07 $\pm$ 0.03	0.213 $\pm$ 0.004
GE	0.78 $\pm$ 0.01	0.210 $\pm$ 0.003	2.00 $\pm$ 0.04	0.213 $\pm$ 0.006
Sites				
AB	0.92 $\pm$ 0.01	0.207 $\pm$ 0.003	2.21 $\pm$ 0.07	0.230 $\pm$ 0.009
HH	0.69 $\pm$ 0.01	0.197 $\pm$ 0.003	1.76 $\pm$ 0.04	0.199 $\pm$ 0.005
GE-1989	0.70 $\pm$ 0.01	0.201 $\pm$ 0.003	2.02 $\pm$ 0.04	0.239 $\pm$ 0.005
GE-Best	0.72 $\pm$ 0.01	0.177 $\pm$ 0.003	2.06 $\pm$ 0.04	0.202 $\pm$ 0.006
GE-Far	0.75 $\pm$ 0.01	0.199 $\pm$ 0.003	1.89 $\pm$ 0.04	0.207 $\pm$ 0.006

## DISCUSSION

This experiment was based on the transfer of 4 local stocks of mussels to 5 different sites in the Magdalen Islands (Southern Gulf of St. Lawrence). The 20 stock  $\times$  site combinations were followed from June 1990 to November 1991 during the ice-free periods. It clearly showed that seed stocks coming from closely neighbouring areas could provide highly variable performances when placed in identical environmental conditions. A local seed stock highly resistant to summer mortality was found. Conversely, the performances obtained at all the grow-out sites were usually comparable. Data on survival rates, growth rates, biomasses and condition indices provided useful information for a better understanding of the summer mortality phenomenon and its causes.

### Survival

The Amherst Basin (AB) stock was highly resistant to the summer mortality since 82% of these mussels were still alive at the end of the experiment. The Great Entry (GE) stock experienced heavy losses during both years and only 11% survived through November 1991. These results corroborated our previous findings that the AB stock had a high survival and the GE stock a low survival (Myrand 1990). Probably one of the most interesting results came from the Bay of Pleasant (BP) stock. In 1990, the 1-yr-old mussels had a survival rate as high as the AB stock but the 2-yr-old individuals were decimated by a heavy summer mortality in 1991. Consequently, their cumulative survival after 2 years was as low as for the GE stock. This mortality occurred at all the experimental grow-out sites more than 21 months after the transfer of the mussels. It is therefore highly unlikely that it could be attributed to a factor acting at the initial site and causing a spurious stock effect. Johannesson et al. (1990) also observed the high sudden death of mussels long after their transfer, e.g., 16 months. Our results also indicated that the survivors of a preceding summer mortality episode cannot be considered as "resistant mussels" as they can have heavy losses in subsequent years.

The survival of mussels from each stock was usually similar at all the experimental sites. This confirmed our preliminary results

(Myrand 1990). Mallet et al. (1990) and Sephton et al. (1993) also found that survival was comparable at their experimental sites in Prince Edward Island (Gulf of St. Lawrence). In all these studies, the sites were dispersed over a relatively small geographic area. It was suggested that the comparable survival probably reflects the general similarity of the environments (Sephton 1991). Comparable temperature profiles at our grow-out sites (Fig. 5) are in agreement with this suggestion.

As stated in other studies (Dickie et al. 1984, Mallet et al. 1987, Mallet and Carver 1989, Mallet et al. 1990, Sephton 1991, Fuentes et al. 1992.), survival was more influenced by a genetic component (stock) than by an environmental component (site). This result is even more striking in our study given the limited range covered by the experimental sites: the stock factor alone explained 92% of the variance of the cumulative survival rate compared to less than 2% for the site component. Genetic differences had been measured directly in the mussels of the Magdalen Islands. Analysis of the mitochondrial-DNA showed that the GE and the BP stocks had very similar profiles which were highly different from the AB mussels (Dodson 1993). However, these differences could not be attributed to the possible belonging of the stocks to one or the other species of blue mussels found concomitantly in eastern Canada, *Mytilus trossulus* and *M. edulis*, since only the latter is present in the Magdalen Islands (Sévigny 1993). Taken together these results support the suggestion of Mallet et al. (1987) that the mortality among stocks is linked to genetic differences.

Dickie et al. (1984) and Mallet et al. (1987) proposed that resistant stocks came from more "stressful" environments which they defined arbitrarily as the sites where the experimental stocks pooled together had an overall low survival rate. Considering the comparable survival at all the experimental sites, it is difficult to invoke some adaptation to stressful conditions by the AB stock as an explanation of its high resistance. The high growth rates, biomasses and condition indices of the mussels from all stocks kept at the AB grow-out site also do not indicate a stressful environment.

Both years the summer mortality occurred in July–August. Previous studies showed that it usually happens between late July and mid August (Bergeron 1991, Myrand 1991a). This is consistent

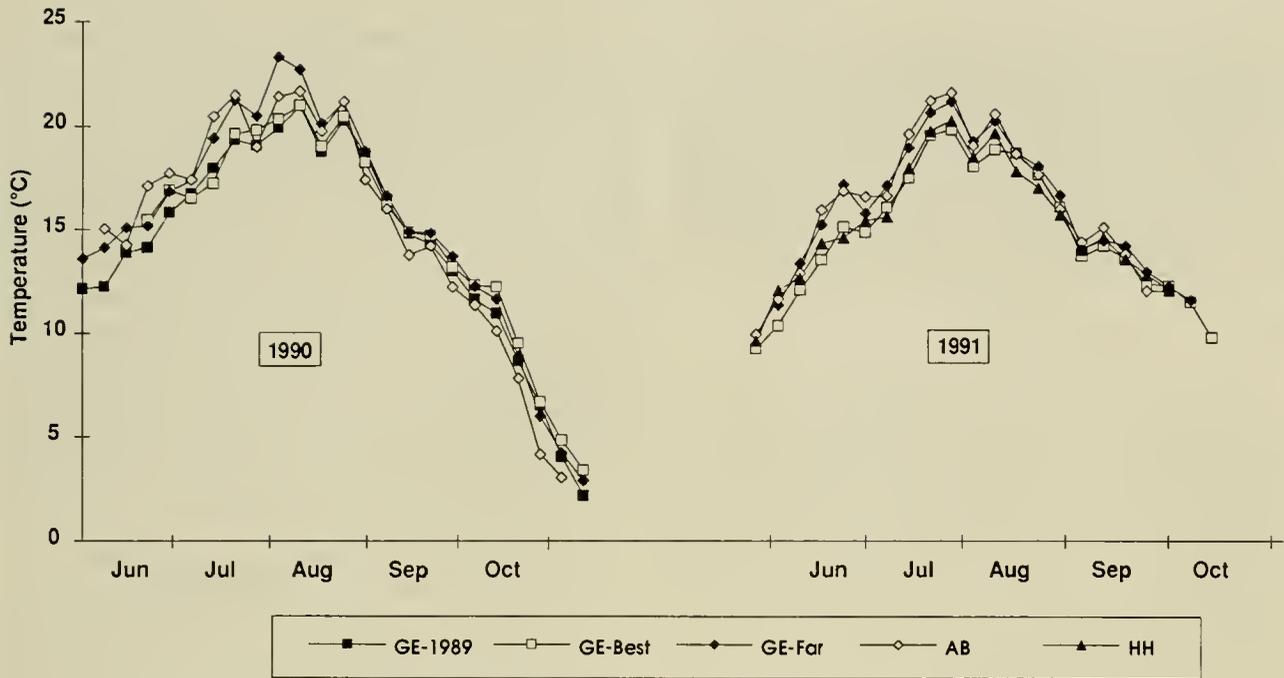


Figure 5. Mean weekly temperature at the experimental sites during the ice-free periods in 1990 and 1991.

with previous observations in the Maritimes (Freeman and Dickie 1979, Mallet et al. 1990) and on the Pacific coast (Jamieson 1991).

#### Growth

The survivors of the decimated BP and GE stocks attained a shell length comparable to the resistant AB stock despite the initial differences in the stocks' mean size (23.0 to 28.7 mm or 24.7%) and their subsequent differential survival. The difference even narrowed to 9.1% (47.0 to 51.3 mm) in November 1990 and 4.4% (63.2 to 66.0 mm) in November 1991. Mallet (1991) also noticed that mussels that could withstand the summer conditions were able to grow very well. From this parameter alone, surviving mussels seemed in good condition. However, we must be cautious when comparing shell lengths since shell growth is uncoupled and precedes the growth of soft tissues (Hilbish 1986). Furthermore equal shell lengths do not necessarily translate into equal tissue masses if, for example, the stocks' rate of tissue replenishment after spawning is different. Caution is justified here since the comparison of the allometric relationships obtained from the experimental combinations showed that similar shell lengths corresponded to different tissue masses.

Mussels from the AB stock had the highest growth rate in 1990 and one of the best in 1991. Thus, these mussels, particularly the 1-yr-old individuals, were able to take advantage of the ambient resources for their growth. In fact, the 1-yr-old AB mussels have a lower metabolism than the GE mussels (R. Tremblay and H. Guderley, Université Laval, Québec, pers. comm.). The AB mussels could therefore invest more energy in growth than the GE mussels because of their lesser requirements. The survival of the BP stock was as high as the AB stock in 1990 but its growth rate was the lowest with the GE stock. Therefore these mussels were apparently not in good condition despite their high survival. Their

heavy losses in 1991 coincided also with the lowest growth rate among the stocks.

In 1990, the surviving mussels from the cages exhibiting substantial mortality tended to have a lower growth rate. Sephton and Bryan (1991) and Mallet and Carver (1993) also noted the same tendency but expressed it otherwise: faster-growing mussels have lower risks of dying than slower-growing mussels. The relation was less clear for the 2-yr-old individuals in 1991 since the growth rate was rather low and similar for most of the stocks.

The growth rate of the mussels kept at the various sites was not very different over the entire experimental period and is probably explained by the closeness of the sites and the general similarity of their environments. Various authors (Dickie et al. 1984, Widdows et al. 1984, Mallet et al. 1987, Mallet and Carver 1989, Kautsky et al. 1990, Mallet et al. 1990, Sephton 1991, Fuentes et al. 1992, Stirling and Okumus 1994) have stressed the importance of environmental factors compared to genetic factors in determining growth. Skibinski and Roderick (1989) even stated that no more than a few percent of the variation of growth could be attributed to populations or genetic differences. In this study, the influence of the sites seemed only slightly higher than the stocks according to the range of the values obtained:  $0.068\text{--}0.078\text{ mm} \cdot \text{day}^{-1}$  for the sites vs.  $0.070\text{--}0.077\text{ mm} \cdot \text{day}^{-1}$  for the stocks.

#### Masses and Condition Indices

Both years, the dry mass of tissue was almost similar for the AB, HH and GE stocks despite their highly differential survival rate. Their condition index was also comparable. Thus, the survivors from the HH and GE stocks seemed in good condition, at least if we assume that the AB mussels were. The high values for the AB stock were in agreement with their higher growth rate and their lower metabolic needs. Conversely, the BP mussels had seemingly greater difficulty converting ambient resources into flesh since their dry mass of tissue and their condition index were

the lowest in 1990 and 1991. This is indicative of the poor condition of the BP mussels in spite of their high survival in 1990. These low values are consistent with their lower growth rates.

Both years, mussels from the AB site provided the best results in terms of dry mass of tissue and condition index. Data from the mussels kept at the 3 GE sites were more difficult to interpret since the dry mass of tissue was quite similar but the mass of their shells differed markedly (3.53–4.12 g in 1990 and 8.52–10.16 g in 1991), resulting in different condition indices.

#### *Possible Causes of Summer Mortality*

Although this experiment was not designed specifically to determine the causes of the summer mortality phenomenon, it does provide evidence for testing certain hypotheses proposed by various authors. First, summer mortality in the Magdalen Islands is not linked to any pathogen according to the specimens examined over the recent years (S. McGladdery, Dept. Fisheries and Oceans, Moncton, pers. comm.) or in the past (Poirier and Myrand 1982). The same opinion prevails for the summer mortality observed elsewhere in the Maritimes (Mallet 1991, Sephton et al. 1993) and on the West Coast (Jamieson 1991).

Food depletion as proposed by Incze et al. (1980) is not directly involved since the growth rates and the condition indices observed in this study were not indicative of starvation. For example, growth rates were comparable or higher than those reported in other studies from the Maritimes (Mallet 1991, Sephton 1991, Mallet and Carver 1993), and the condition index of the decimated GE stock was higher than the resistant AB stock in 1990. If starvation was to be considered, we would have observed a site effect rather than an important stock effect. In fact, food is observed to increase in August (Mayzaud and Souchu 1991, Myrand 1991b) when summer mortality occurs. Mallet (1991) also argued against the hypothesis of low food levels to explain increased mortality in Prince Edward Island.

Freeman and Dickie (1979), Incze et al. (1980), Mallet (1991) and Sephton (1991) proposed that high temperature may play a major role on summer mortality. However, summer mortality in the Magdalen Islands is difficult to link with stressing temperature ( $>20^{\circ}\text{C}$ ) as did Incze et al. (1980). Between 1 and 68 Day-Degrees  $> 20^{\circ}\text{C}$  were recorded at the experimental sites in 1990 and 1991 (Fig. 5) with comparable survival among the sites and among the years. Likewise Mallet et al. (1990) did not find any differential survival at their experimental sites although the stressing regime varied between 7 and 92 Day-Degrees  $> 20^{\circ}\text{C}$  (Mallet 1991). In addition, summer mortality of mussels was observed previously in the Magdalen Islands even when water temperatures were not warmer than  $18^{\circ}\text{C}$  (Myrand 1991a). Jamieson (1991) also reported summer mortality in the cool waters of British Columbia.

Carver and Mallet (1991) suggested that summer mortality could rather be related to differential stocks' tolerance to high temperature. This hypothesis does not help to understand the differential survival of the AB, HH and GE stocks since these mussels were presumably adapted to the environmental conditions of their sites of origin, and thus to similar thermal regimes (Myrand 1991b) (Fig. 5). Also the BP stock from the cooler open coast site (Myrand 1991a) was decimated only during the second summer after its transfer although water temperature was higher in 1990. The heavy losses of the 2-yr-old individuals from the GE and HH stocks that survived to the preceding summer mortality episode is

also difficult to match with thermal sensitivity because these mussels would rather have been eliminated in 1990 when temperature was warmer.

Mallet and Carver (1993) and Sephton et al. (1993) proposed that the varying intensity of the summer mortality from one year to the other could be linked to the selective pressure of high temperature acting on spat (post-larvae). According to this hypothesis the spat with low thermal tolerance would be eliminated shortly after settlement during the warm period of the summer. Consequently, a "cool" summer should characterize the year preceding a major summer mortality of 1-yr-old mussels in order to minimize the initial thermal selection during the period of spat collection. The decimated GE and HH stocks were submitted to 250 and 247 Day-Degrees  $> 15^{\circ}\text{C}$  at their sites of origin in 1989 (unpubl. data) and to 246–382 Day-Degrees  $> 15^{\circ}\text{C}$  at the various grow-out sites in 1990. It is therefore difficult to qualify the summer of 1989 as "cool" when compared to 1990. This hypothesis is not validated by our observations.

Hypotheses based on food depletion, high temperature stress or thermal sensitivity alone cannot explain our results adequately. A factor related to the age of mussels must be involved to explain high losses of the 2-yr-old individuals that survived to the previous summer mortality episode. Their survival (52%) was lower than the 1-yr-old individuals (60%) although environmental conditions were better (cooler temperature). Various authors have insisted on the stress associated with gametogenesis and spawning, and thus on the possible major role of reproduction on mortality (Bayne et al. 1978, Bayne et al. 1980, Perdue et al. 1981, Emmet et al. 1987, Jamieson 1989, Newell and Lutz 1991, Thompson 1991). Thus higher losses of 2-yr-old individuals could be explained by their higher reproductive effort (Worrall and Widdows 1984, Emmett et al. 1987). Mallet et al. (1990) could not link spawning and summer mortality of mussels from Prince Edward Island because the main spawning event occurred in May, e.g., long before the die-off. Mallet (1991) also observed low gonad masses (indicative of a high reproduction output) associated with high survival among certain stocks. However, Shpigel et al. (1992) gave support to the possible link between reproduction and summer mortality by comparing the performances of diploid and triploid Pacific oysters. The present study did not provide precise information on reproduction but results can be explained in light of this hypothesis.

Considering the previous information, the following is proposed to explain the observations of the present study in the Magdalen Islands. Summer mortality could be related to the stress accompanying the second spawning event of the cultured mussels which occurs usually during the second half of July (Poirier and Myrand 1981, Myrand 1991b). The timing of the mortality observed in this study added to this hypothesis. A second spawning preceding the summer mortality of 2-yr-old GE mussels has already been noticed (Myrand et al. 1993). Newell et al. (1982) showed that food availability has a major influence on the reproductive cycle. Since environmental conditions are known to be comparable in the lagoons of the Magdalen Islands (Myrand 1991b), it is hypothesized that gametogenesis and spawning are relatively well synchronized at all sites. Profiles of the mussels' meat yield from the HH and the GE lagoons over the years support this view (Myrand 1991b). Consequently, if mortality is considered a reproductive cost (Bayne 1984, Thompson 1991), it is no surprise that it happened at the same time and with comparable intensity at all sites. This second spawning event is followed by the

warmest period of the year. That factor imposes an additional stress on the exhausted mussels which are more sensitive to high temperature (Bayne et al. 1976). This stressful condition amplifies the reproductive cost (Bell and Koufopanou 1986, Shpigel et al. 1991).

Maybe the observed differences among the stocks could be explained by differential investment in reproduction and/or differential potential of replenishment after spawning. Individuals that channel more energy in their reproduction are more susceptible to summer mortality as they become more exhausted after spawning. Furthermore, mussels with higher metabolic demands have less chance to get through the difficult post-spawning period since they need more energy for their maintenance. It is not known if the AB mussels have a lower reproductive effort than the GE mussels but they have lower metabolic needs (R. Tremblay and H. Guderley, Université Laval, Québec, pers. comm.). In the event of a comparable reproductive effort, the AB mussels could survive the summer period because they could replenish themselves more easily after spawning.

Results from the BP stock are more difficult to explain. This stock may be less tolerant to the lagoons' high temperature because of its origin. Both years, it showed signs of stress when compared to the other stocks. The small 1-yr-old mussels had a low reproductive effort and thus could survive through the first

year although being stressed. Their higher reproductive effort during the second summer was probably highly detrimental to them. Their losses were the highest recorded during the experiment, probably as a consequence of the compound effect of their reproductive effort and their low tolerance to high temperature.

Further studies are needed to validate the hypothesis that summer mortality in the Magdalen Islands should be considered as a reproductive cost. Reproductive effort, energetic content and metabolism of one- and two-year-old mussels from stocks with different survival rates should be compared.

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## ULTRASTRUCTURE OF SPERMATOOZOA IN INDUCED TETRAPLOID MUSSEL *MYTILUS GALLOPROVINCIALIS* (LMK.)

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**ABSTRACT** Ultrastructure of spermatozoa in artificially induced tetraploid mussels, *Mytilus galloprovincialis*, was examined by light and electron microscopy and compared to that in normal diploid mussels. Sixteen tetraploid mussels were examined. Eight were found to be males, seven had spent or undeveloped gonads for which sex could not be determined, and one was an hermaphrodite. Ultrastructure of spermatozoa from tetraploids was similar to diploids, but there were some differences. Differences included the number of mitochondria (5 to 7 in tetraploids compared to only 5 in diploids) and the presence of abnormal acrosomes (2.4% in tetraploids, 0% in diploids). The mean size of spermatozoa in tetraploids was larger than that from diploids: acrosome length (4.2  $\mu\text{m}$  vs. 3.0  $\mu\text{m}$ ), nuclear width (2.1  $\mu\text{m}$  vs. 1.8  $\mu\text{m}$ ), nuclear length (2.1  $\mu\text{m}$  vs. 1.9  $\mu\text{m}$ ), and flagellar length (73  $\mu\text{m}$  vs. 63  $\mu\text{m}$ ). The DNA content of spermatozoa in tetraploids was about 2 times that of spermatozoa from diploids. Oocytes were observed in the hermaphroditic tetraploid, but only in very low numbers. These findings indicate spermatozoa will be capable of fertilization, and therefore, useful in the production of triploids by crossing to normal haploid oocytes.

**KEY WORDS:** *Mytilus galloprovincialis*, tetraploid, spermatozoa, ultrastructure

### INTRODUCTION

Induced triploid bivalves are useful in aquaculture because the reduced gamete output improves meat quality and growth (Allen and Downing 1991, Akashige and Fushimi 1992). Triploidy is usually induced by preventing meiosis II of fertilized eggs. Several procedures (chemical treatment, hydropressure, and temperature shock) for the induction of triploidy in bivalves have been successful (Allen 1987, Beaumont and Fairbrother 1991). However, the survival of larvae in treated groups has been found to be lower than the controls (Tabarini 1984, Downing and Allen 1987, Wada et al. 1989, Utting and Child 1994). Additionally, the percentages of triploid induction were not always 100% for these studies.

A novel method to increase triploid induction rates and survival would be the use of multiple-genome gametes. The crossing of gametes from tetraploids and diploids has been shown to produce all triploid populations in brown frog (Kawamura et al. 1963) and rainbow trout (Chourrout et al. 1986). This indicates that the gametes from tetraploids contained a diploid genome complement.

In bivalves, viable spermatozoa with a DNA amount 1.5 times that of haploid spermatozoa were obtained from triploid Pacific oysters (Akashige 1990). Eggs fertilized with these sperm were aneuploid, containing 2.5 times the DNA level of haploid sperm (Akashige 1990). Oocytes from triploid Japanese pearl oysters fertilized with spermatozoa from diploids yielded some juveniles with either a diploid or triploid genome (Komaru and Wada 1993). This indicated that a small proportion of eggs contained twice the genome complement. Therefore, the use of "diploid gametes" from tetraploids for crossing with haploid gametes from diploids may be an efficient procedure for the production of triploids. We have previously reported the successful induction of tetraploidy in mussels (Scarpa et al. 1993). In the present study, gamete production and ultrastructure in tetraploid mussels were compared to that of diploid mussels by light and electron microscopy in order to delineate their possible performance characteristics.

### MATERIALS AND METHODS

#### Organisms

The parental mussels (*Mytilus galloprovincialis* Lamarck) used to produce the tetraploid and diploid mussels examined in the present study were collected in January, 1993 from a natural population found in Gokasho Bay, Mie Prefecture, Japan (Scarpa et al. 1993). Mussels were spawned the same day as collection and tetraploidy was induced by inhibiting meiosis in fertilized eggs of mussels with cytochalasin B (Scarpa et al. 1993). After 3 months of controlled culture the spat were transferred to a pearl net and hung from a raft in Gokasho Bay.

Mussels were sampled in March and April, 1994. The mean shell height and standard deviation of mussels used were: tetraploid (3.13  $\pm$  0.39 cm, n = 11), diploid (3.70  $\pm$  2.06 cm, n = 8) in March and tetraploid (3.48  $\pm$  0.58 cm, n = 5), diploid (4.90 and 3.80 cm, n = 2) in April. Large mussels were not sampled in this study as Scarpa et al. (1993) reported that tetraploids were only found in the smaller size grouping.

#### Histological Procedures

Gonadal tissue was fixed with 4% glutaraldehyde in cacodylate buffer (pH 7.5) (Komaru et al. 1994) for the March sample and Bouin fixative for the April sample. Tissue samples were then dehydrated through a graded ethanol series and embedded in paraffin. Sections (6  $\mu\text{m}$ ) were cut, placed on glass slides, and stained with acid-haemalaun and eosin.

Tissue samples fixed with 4% glutaraldehyde in cacodylate buffer were also prepared for electron microscopy following the procedure by Komaru et al. (1994). Small pieces (1-2 mm<sup>2</sup>) of gonadal tissue were postfixed with 1% osmium tetroxide, dehydrated through a graded ethanol series and embedded in epoxy resin Quetol 812 (Nisshin E.M. Co., Tokyo, Japan). Ultrathin

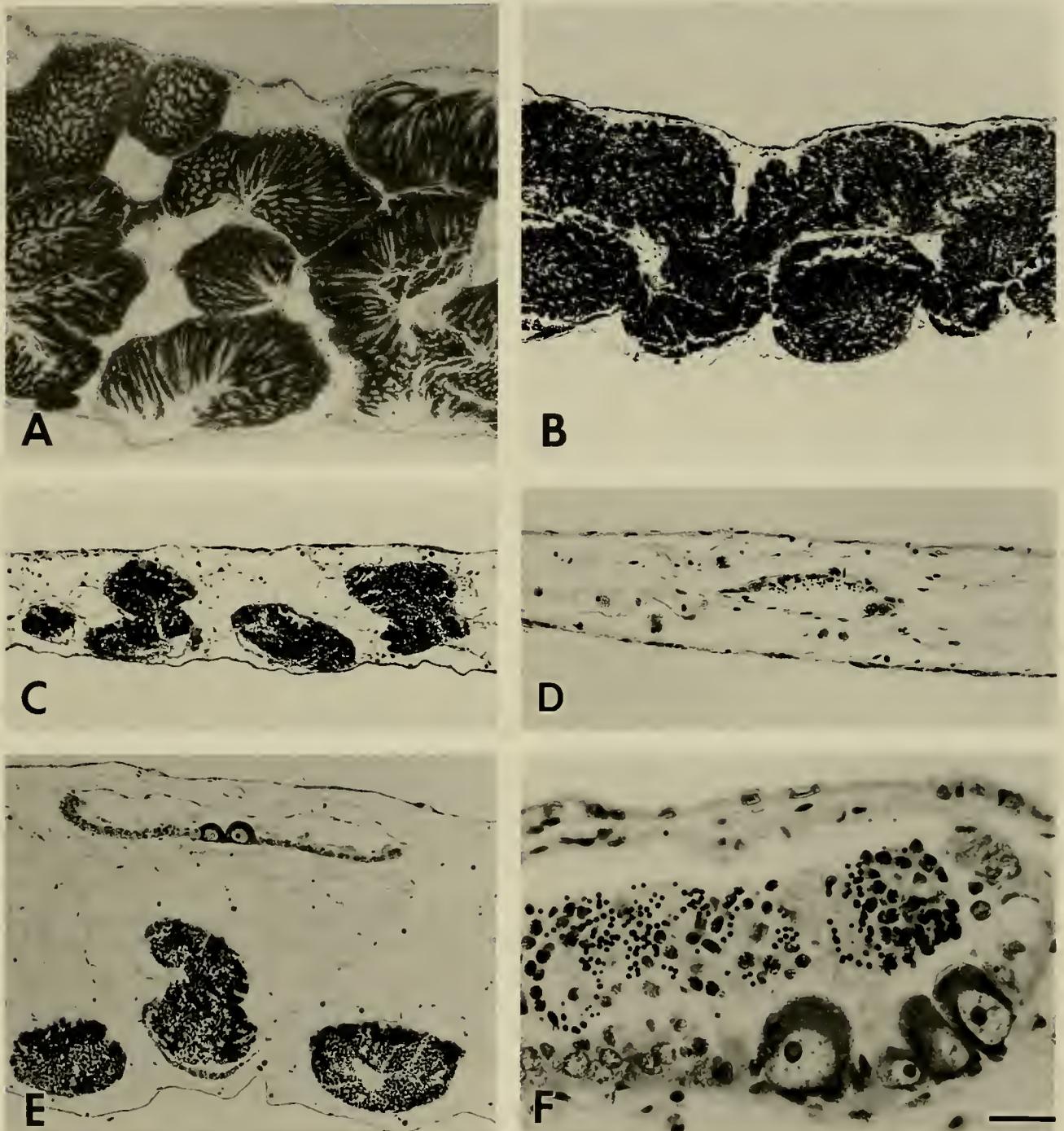


Figure 1. Light micrograph of gonadal tissue section from a diploid (A) and tetraploid (B–F) mussel, *Mytilus galloprovincialis*. A: Diploid mature testis. B,C: Tetraploid mature testis. D: Tetraploid spent testis. E,F: Tetraploid hermaphrodite gonad. Scale: A,B,C,E: 100  $\mu\text{m}$ , D: 50  $\mu\text{m}$ , F: 25  $\mu\text{m}$ .

sections were stained with uranyl acetate and lead citrate, and observed using a JEOL 1200EX transmission electron microscope.

#### DNA Quantification

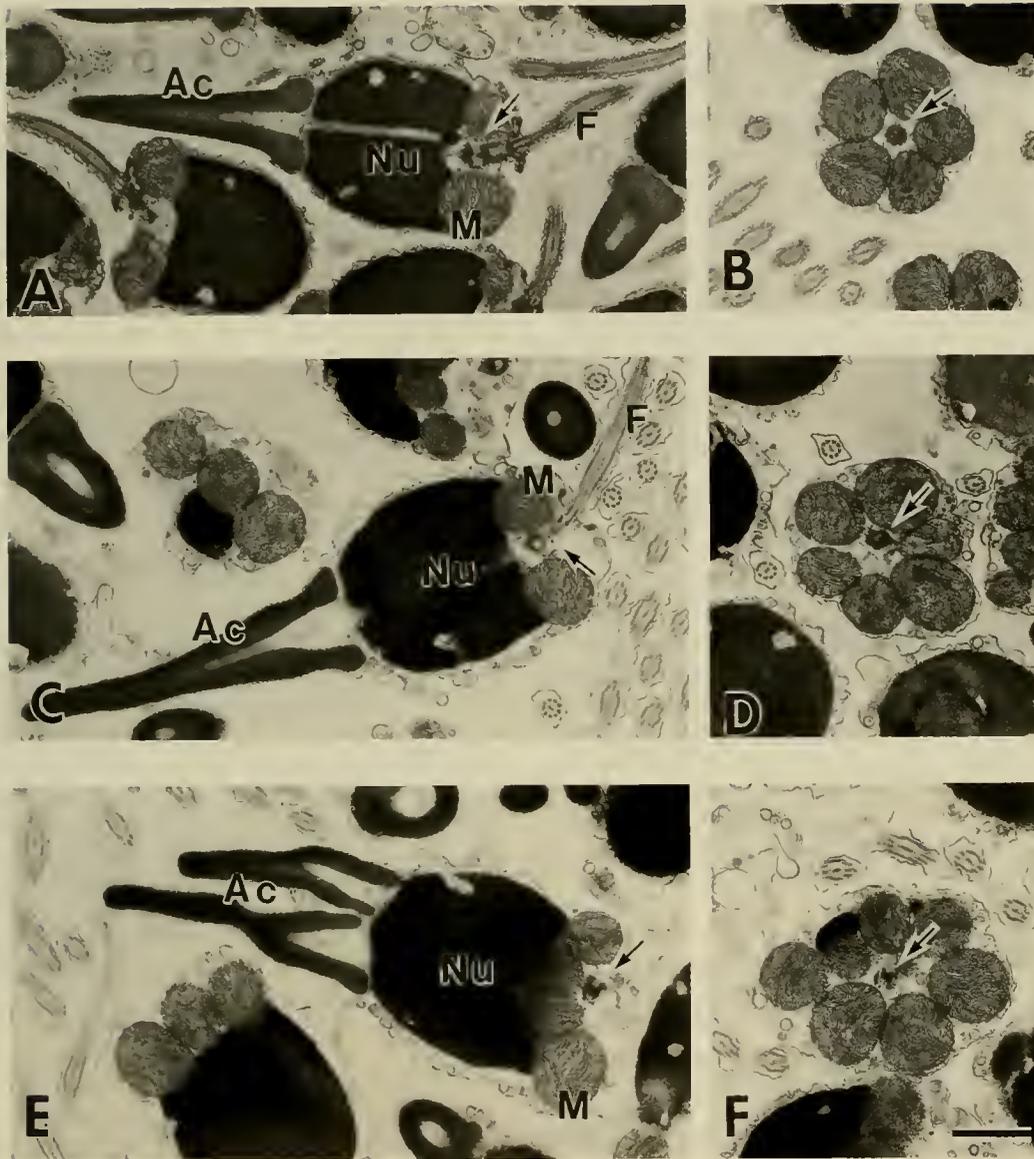
Ploidy of mussels was determined by DNA microfluorometry following the procedure of Komaru et al. (1988). Gill cells were dissociated from glutaraldehyde-fixed materials by mincing with scissors in 50% acetic acid, dropped on a prewarmed glass slide and air dried. The cells were stained with the DNA specific dye

DAPI and the fluorescence intensity per nucleus ( $n = 30$ ) was measured by microfluorometry. The DNA content of spermatozoa ( $n = 100$ ) was also estimated using this procedure.

#### Spermatozoa Measurements

Nuclear width, nuclear length, and acrosome length were measured from the paraffin embedded material stained with acid-haemalaun and eosin.

Spermatozoa from gonads fixed with 4% glutaraldehyde were



**Figure 2.** Electron micrograph of spermatozoa in diploid (A,B) and tetraploid (C–F) mussel, *Mytilus galloprovincialis*. A: Longitudinal section through spermatozoon in diploid. B: Transverse section through midpiece in diploid. C: Longitudinal section through spermatozoon in tetraploid. D,F: Transverse sections through midpiece with 6 (D) and 7 (F) mitochondria (M) in tetraploids. E: Longitudinal section through abnormal spermatozoon with 2 acrosomes. Acrosome = Ac, Nucleus = Nu, Flagellum = F. Arrows indicate centrioles. Scale: 1  $\mu\text{m}$ .

dispersed by mincing with scissors in 50% acetic acid, dropped on a prewarmed glass slide and air dried. Unstained preparations of dispersed spermatozoa were observed by phase contrast microscopy. Flagella length was measured with an ocular micrometer. The percentage of spermatozoa with 2 acrosomes was also estimated. The mean size of spermatozoa components ( $n = 30$ ) between tetraploids and diploids was statistically analyzed using Student's *t*-test (Sakuma 1964).

The number of mitochondria per spermatozoa were counted on TEM negatives showing transverse sections of the midpiece.

## RESULTS

### Gamete Production

In the March sample, 8 diploids and 11 tetraploids were examined. Among the diploids, three were mature males (Fig. 1A),

one was a mature female, and four had spent gonads. Among tetraploids, four contained spermatozoa (Fig. 1B,C) but were less prolific compared to the diploids (Fig. 1A). The other 7 tetraploids had spent gonads, but two of these seven were identified as male because of residual spermatozoa (Fig. 1D). In the April sample, one of 5 tetraploids was an hermaphrodite, two were identified as male because of residual spermatozoa, and the other two had spent gonads.

In the hermaphrodite tetraploid, oocytes and spermatozoa were usually observed in different acini (Fig. 1E). Acini filled with spermatozoa predominated, but spermatozoa and oocytes were observed occasionally in the same acini (Fig. 1F). The oocytes had a large nucleus, prominent nucleoli, and well developed cytoplasm, although the number of oocytes were few. The means  $\pm$  sd of the long and short axis of 10 oocytes from the hermaphrodite were  $68.5 \pm 4.0 \mu\text{m}$  and  $52.6 \pm 7.2 \mu\text{m}$ , respectively. In com-

TABLE 1.

Size (mean  $\pm$  standard deviation) of spermatozoa components from 2 tetraploid and 1 diploid mussel in March, 1994.

	Tetraploid		Diploid
	No. 1	No. 2	
Length of			
Acrosome ( $\mu\text{m}$ )	4.27 $\pm$ 0.30*	4.22 $\pm$ 0.22*	2.98 $\pm$ 0.08
Width of Nucleus ( $\mu\text{m}$ )	2.16 $\pm$ 0.06*	2.14 $\pm$ 0.06*	1.78 $\pm$ 0.07
Length of Nucleus ( $\mu\text{m}$ )	2.06 $\pm$ 0.06*	2.04 $\pm$ 0.05*	1.85 $\pm$ 0.06
Length of			
Flagellum ( $\mu\text{m}$ )	72.3 $\pm$ 2.3*	75.1 $\pm$ 2.3*	63.0 $\pm$ 1.9

\* Significantly different ( $P < 0.01$ ). Number of sperm measured was as follows; tetraploid: 30, diploid: 30.

parison, the means of the long and short axis of 10 oocytes in one mature diploid were  $60.1 \pm 2.6 \mu\text{m}$  and  $45.2 \pm 4.4 \mu\text{m}$ , respectively.

#### Ultrastructure of Spermatozoa

General ultrastructural morphology of spermatozoa produced by tetraploids (Fig. 2C,D) was almost identical to that of spermatozoa produced by diploids (Fig. 2A,B), except for the number of mitochondria and increased size of flagellum, acrosome, and nucleus (Table 1). Spermatozoa produced by tetraploids (Fig. 2C,D) were composed of an elongated acrosome ( $4.2 \mu\text{m}$ ), an electron dense nucleus ( $2.2 \mu\text{m} \times 2.1 \mu\text{m}$ ), spheroid mitochondria and a flagellum ( $74 \mu\text{m}$ ). These measured parameters were all significantly larger ( $P < 0.01$ ) in the tetraploids compared to the diploids.

The acrosome was elongated and conical, composed of an outer layer with electron dense material and inner layer of electron lucent material. The nucleus was filled with fine electron dense materials and was barrel shaped. As shown in Table 2 the number of mitochondria per spermatozoa produced by tetraploids ranged from five to seven (Fig. 2D,F) with a modal number of six, while the number of mitochondria in diploids was always five (Fig. 2B). Centrioles were observed in the mitochondrial ring (Fig. 2C,D) of tetraploids.

In testes from tetraploid most spermatozoa were not grossly different from those in diploids (Fig. 2C), but abnormal spermatozoa with 2 acrosomes were found occasionally (Fig. 2E). The percentage of spermatozoa with 2 acrosomes was 1.7% and 3.0% in 2 different tetraploids examined in the March sample (Table 3).

Figure 3 shows the DNA content of spermatozoa from tetraploids and diploids. Spermatozoa could be differentiated from other cell types because of the elongated nucleus, ensuring that only spermatozoa DNA levels were measured. The mean DNA

TABLE 2.

Frequency distribution of the number of mitochondria in spermatozoa observed in tetraploid and diploid mussel in March, 1994.

Number of Mitochondria	Tetraploid (%)	Diploid (%)
4	0	0
5	25	100
6	50	0
7	25	0

Number of sperm observed was as follows; tetraploid: 20, diploid: 11.

TABLE 3.

The percentages of spermatozoa with abnormal acrosome produced by 2 tetraploid (No. 1, No. 2) and 2 diploid (No. 1, No. 2) mussels fixed in March, 1994.

	Tetraploid		Diploid	
	No. 1	No. 2	No. 1	No. 2
Abnormal Acrosome (%)	1.7	3.0	0	0
Normal Acrosome (%)	98.3	97.0	100	100
Number of Sperm Observed	423	427	328	253

content of spermatozoa from 2 tetraploids was about 2 times (2.10 and 1.95) that of spermatozoa from diploids.

#### DISCUSSION

Although gamete production and ultrastructure have been examined in a number of bivalves (Popham 1979, Hodgson and Bernard 1986, Dorange and Le Pennec 1989, Thielly et al. 1993), it is only recently that gamete ultrastructure in a polyploid bivalve has been reported (Komaru et al. 1994). In the present study, male tetraploids predominated in the sample taken; therefore, only spermatozoa ultrastructure could be observed and compared in detail.

Spermatozoa produced by tetraploid mussels were composed of an acrosome, nucleus, mitochondria, and flagellum and their morphology was almost identical to that of diploid mussels reported (Hodgson and Bernard 1986). Judging from ultrastructure, sperm from tetraploid mussels should be functional. Chourrout et al. (1986) reported that in trout the low fertilizing ability of spermatozoa produced by tetraploids was due to the difficulty of sperm penetrating the micropyle. In mussels this should not be of consequence since mussel sperm enter into the egg cytoplasm follow-

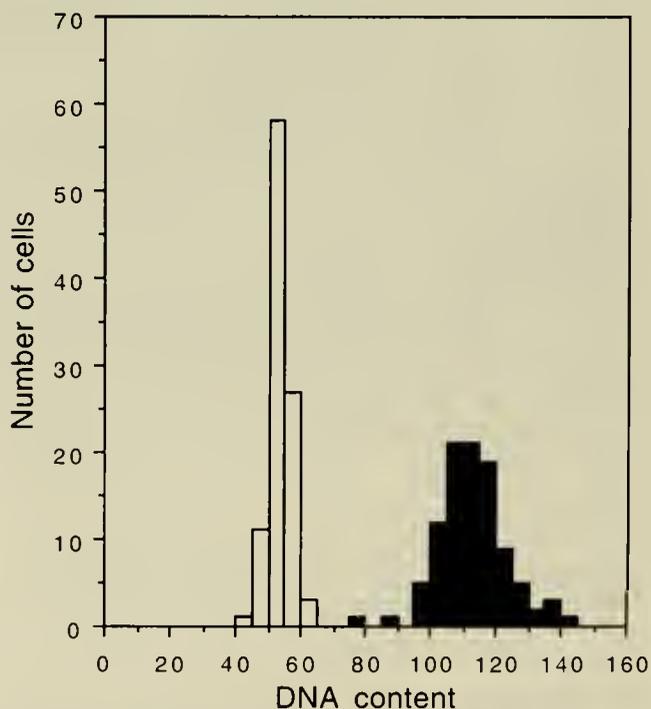


Figure 3. Relative DNA content of spermatozoa as measured by microfluorometry from diploid (white bars,  $n = 100$ ) and tetraploid (black bars,  $n = 100$ ) mussel *Mytilus galloprovincialis* in March 1994.

ing an acrosome reaction with the egg membrane (Nijima and Dan 1965). It should be examined whether the acrosome reaction of sperm from tetraploid mussels would be adequate and normal for penetration.

In all measured parameters, the size of spermatozoa in tetraploids was larger than that of diploids. The nuclear size may be increased due to the increased DNA content as shown by DNA microfluorometry. Flagella length and acrosome length in tetraploids were significantly larger. How the increased DNA content may give rise to a larger acrosome and longer flagellum is not apparent from our study.

It was interesting to observe that the number of mitochondria in spermatozoa from tetraploids was greater by one to two as compared to spermatozoa of diploids. In diploids, the number of mitochondria was five, as previously reported (Hodgson and Bernard 1986). It has been previously suggested that motility of spermatozoa produced by triploid Pacific oysters may be reduced, because the head was significantly larger (compared to spermatozoa from diploids) and the number of mitochondria was the same (Komaru et al. 1994). Sperm from tetraploids with only 5 mitochondria, which is the number found in sperm from diploids, may have reduced motility compared to the sperm of diploids. However, the increased number of mitochondria in the majority of sperm from tetraploids may negate the size increase with resultant motility similar to sperm from diploids.

Although differences of spermatozoa ultrastructure between tetraploids and diploids were primarily size related, another difference was the production of abnormal spermatozoa with 2 acrosomes. The spermatozoa produced by tetraploids most likely have 2 sets of chromosomes and the formation of 2 acrosomes may be related to this. The final morphogenetic process may sometimes be abnormal due to 2 sets of genes being expressed that regulate acrosome formation.

Spermatozoa produced by tetraploids will be useful for producing triploid and other polyploid levels if they contain 2 euploid

chromosome sets. In the present study this was indicated indirectly by DNA microfluorometry. The induction of triploidy is usually achieved by preventing polar body formation in fertilized eggs from diploids (Allen 1987, Beaumont and Fairbrother 1991). Treatments used to induce triploidy generally reduce larval survival compared to untreated eggs and the proportion of triploid organisms is often less than 100% (Tabarini 1984, Downing and Allen 1987, Wada et al. 1989, Scarpa et al. 1994, Utting and Child 1994). If spermatozoa with a diploid genome were available the production of 100% triploid population could be easily achieved by inseminating normal haploid eggs with these "diploid sperm". Recently, this has been achieved in the Pacific oyster (Dr. Ximing Guo, pers. comm.). Additionally, if second polar body formation was inhibited in such a cross tetraploidy could theoretically be induced (Kawamura et al. 1963, Chourrout et al. 1986). A similar technique was used to produce tetraploids by inhibiting polar body 1 formation in eggs from triploid oysters fertilized with normal haploid sperm (Guo and Allen 1994).

In the present study, well-developed oocytes in an hermaphrodite tetraploid were also observed. A cross between oocytes and spermatozoa produced by tetraploids would also yield tetraploids if the oocytes had a diploid genome. Oocytes containing 2 genomes have been produced by triploid pearl oysters as indicated by the ploidy of surviving juveniles (Komaru and Wada 1994). "Diploid sperm" may be useful not only for the induction of an all triploid or polyploid population but for producing androgenetic organisms by crossing diploid sperm and genetically inactivated oocytes (Kusunoki et al. 1994).

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## SETTLEMENT OF *PINCTADA MAXIMA* (JAMESON) AND OTHER BIVALVES ON ARTIFICIAL COLLECTORS IN THE TIMOR SEA, NORTHERN AUSTRALIA

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**ABSTRACT** The type, abundance and growth of tropical bivalves that settled on artificial collectors in the Timor Sea off northern Australia was investigated. Collectors made of used monofilament net were set and retrieved over 5 periods between June 1993 and June 1994. A large number of bivalves were collected, encompassing 47 species from 14 families. Thirteen species from 6 families comprised over 90% of the total numbers, and included various species of commercial importance such as pearl oysters, fan shells and edible oysters. The silverlip pearl oyster, *Pinctada maxima*, was the most valuable of these and accounted for 2.4% of total numbers. It had highest settlement rates during the wet season, between October to January, and a mean estimated growth rate of 5.8 mm · month<sup>-1</sup>. Settlement rates, examined for commonly collected species, tended to be highest during the wet season. Length-frequencies and growth rates of species with commercial potential are presented. Considering the number and type of species collected, there is potential for use of artificial collectors in tropical waters either for aquaculture or as a stock assessment tool.

**KEY WORDS:** *Pinctada maxima*, artificial collectors, settlement, growth, bivalves, tropical

### INTRODUCTION

Artificial collectors consisting of used monofilament net in open-weave bags have proved successful for collecting bivalve spat in temperate waters (Brand et al. 1980). They have been used variously to collect bivalve spat for aquaculture (Paul et al. 1981, Rose and Baker 1994), estimate recruitment in wild-stock fisheries (Sause et al. 1987, Young et al. 1988), and monitor early growth and mortality of bivalves (McLoughlin et al. 1988). There is increasing interest in the potential of collectors for capturing tropical species of commercial value, especially the silverlip pearl oyster, *Pinctada maxima* (Jameson), which sustains Australia's pearl culture industry. *P. maxima* has been successfully collected on artificial collectors under conditions of high spat densities such as pearl farms (Scoones 1991) or in hatcheries (Rose and Baker 1994). This success, and anecdotal evidence of settlement of *P. maxima* on nets and ropes left near oyster beds, prompted me to use artificial collectors in the natural environment to determine what commercially important bivalves settled on these collectors, and establish whether or not collectors could be deployed for commercial purposes or as a research tool for stock assessment. This report identifies which bivalve species settled on artificial collectors in the Timor Sea off northern Australia, and describes settlement and growth rates of the most common species. Particular reference is made to *P. maxima* and other bivalve species with commercial potential, including other pearl oysters (Pteriidae), fan or ear shells (Pinnidae) and edible oysters (Ostreidae).

### MATERIALS AND METHODS

Collectors were placed on 2 shoals in the Timor Sea about 30 km west of Bathurst Island (near 129°48'E, 11°39'S) (Fig. 1). The shoals, about 15 km apart, were chosen because they were relatively shallow (24–27 m) and reportedly contained reasonable numbers of *P. maxima*. Both shoals consisted of low profile reef, usually with a shallow layer of coarse sand covering large areas, and were in open water, subject to tidal currents of up to 3 km · hr<sup>-1</sup>.

On each shoal, 4 stations of collectors were placed at 80 m

intervals along a line with marker buoys at either end. Sixteen collectors were placed at each station as illustrated in Figure 2. Each collector, the smallest replicated unit, was constructed from a black polyethylene mesh bag (500 mm × 230 mm with 7 mm mesh) with 30 g (±0.1 g) of used, 50–75 mm mesh, nylon monofilament net placed loosely inside. The open end of each numbered collector was drawn together with a cable-tie that also held a stainless-steel snood to attach the bags to the stations. Collectors were set and collected by divers over 5 periods between June 1993 to June 1994 (Table 1). Four bags (2 from each PVC pipe) were retrieved and replaced from each station after each period. They were brought to the surface, drained in the shade and frozen in sealed plastic bags. In the laboratory, collectors were thawed and weighed. The monofilament was removed from the bags and the bivalves were extracted, identified, counted, and measured (dorso-ventrally) to the nearest mm. The experimental design consisted of 2 replicates from the upper and lower PVC pipes from each of 8 stations (A–H) split between 2 shoals (1 and 2) taken over 5 periods (Jun–Aug, Aug–Oct, Oct–Jan, Jan–Mar, and Mar–Jun).

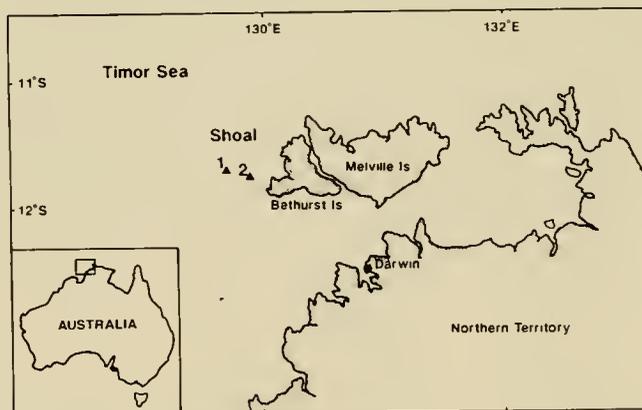


Figure 1. Artificial collectors were placed on 2 shoals in the Timor Sea about 30 km west of Bathurst Island. The shoals, about 15 km apart, were chosen because they were relatively shallow (24–27 m) and reportedly contained reasonable numbers of *P. maxima*.

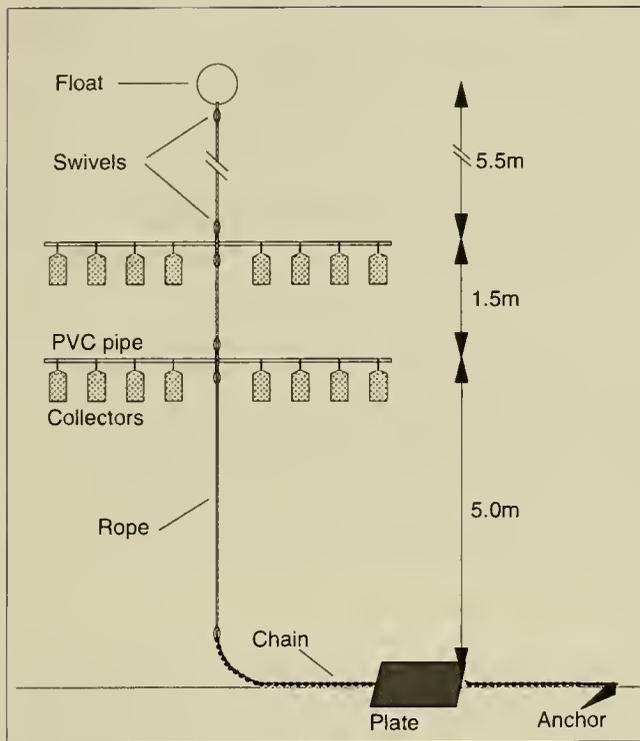


Figure 2. Each station, which incorporated 16 collectors, was constructed from 13 mm silver rope and buoyed 12 m above the bottom by a 5 kg high density polypropylene float. At 2 heights up the rope (5 and 6.5 m from the bottom), a 3 m length of 50 mm PVC pipe was connected horizontally by a central swivel. Eight collectors were attached at 300 mm intervals to each pipe. Each station was anchored via 1.5 m of 10 mm proof coil galvanised chain to a 25 kg steel plate connected to a 10 kg anchor.

Many of the collectors retrieved had been torn open and the monofilaments was missing; these were excluded from any analyses (Table 1). Other collectors were retrieved after longer periods of immersion, but those data are not presented here.

The relative abundance of different species and families was compared. Analyses of growth and settlement rates were undertaken on the 13 most common species. Standardised monthly settlement rates, where:

Settlement rate =

$$\frac{\text{No. of Individuals}}{\text{Standard 30g spat bag}} \times \frac{30}{\text{Period duration}}$$

were averaged, and included undamaged bags on which no individuals were found. Spatial (shoal, station and height) and temporal (period) differences in settlement rates of common species were compared using analysis of variance (ANOVA) and Scheffe's multiple comparison test.

Growth rates were obtained by averaging individual growth rates for each period. They were calculated based on the assumption that the bivalves settled at the middle of each period and are expressed in  $\text{mm} \cdot \text{month}^{-1} \pm \text{standard deviation}$ . Growth rates and length frequency histograms (5 mm length classes) of commercially important species are given for each period.

## RESULTS

The abundances of the different bivalve species and families are summarised in Table 2. During the experiment a total of 1639 bivalves were collected, encompassing 47 species from 14 families. Thirteen species from 6 families comprised over 90% of the total numbers and were included in further analyses. Of these, six have commercial potential, including *P. maxima*, *Pinctada albina*, *Pteria breviaalata*, *Pteria penguin*, *Pinna bicolor*, and *Saccostraea cucullata*. *Pinctada margaritifera*, *Atrina pectinata*, *Pinna incurva*, *Pinna deltodes* and *Saccostraea echinatum* also have commercial potential, but were only caught in low numbers (<10 individuals).

In general, settlement rates showed extreme variation in proportion to the mean and comparisons were therefore performed on log-transformed data. *P. bicolor* had greater settlement on the upper pipes of the stations (Scheffe's  $P < 0.05$ ) but as it was the only species to exhibit a significant difference on this spatial scale, data from each station were pooled for analysis of other spatial effects. Of the 13 common species, only *Dendostrea folium*, *Malleus atrinus* and *P. breviaalata* had significant differences (Scheffe's  $P < 0.05$ ) in settlement rates between shoals (higher on Shoal 1) and only *D. folium* differed between stations. Analysis of the effect of period was consequently based on data pooled for spatial effects.

Pooled over species, the period had a significant effect on settlement rates ( $F_{4,81} = 5.59$ ,  $P < 0.0005$ ), with the trend of

TABLE 1.  
Dates and number of collectors that were set and retrieved for each period.

Date	Period	No. of Collectors				
		Set	Retrieved	Damaged	Analysed	Missing
18/06/93	Jun-Aug	12	9	0	9	3
11/08/93	Aug-Oct	32	31	4	27	1
22/10/93	Oct-Jan	32	16	1	15	16*
22/01/94	Jan-Mar	32	24	5	19	8
21/03/94	Mar-Jun	32	23	7	16	9
06/06/94	Total	140	103	17	86	37

\* The PVC pipes on 2 stations were broken causing the loss of many collectors. Analysis was only conducted on the collectors that had received little damage and had all of the monofilament still intact.

TABLE 2.

List of bivalve species found on collectors set in the Timor Sea for five 2–3 month periods over one year.

Family	Species	Frequency	
		No.	%
Mytilidae		561	34.2
Pteriidae		456	27.8
Pectinidae		219	13.4
Malleidae		129	7.9
Pinnidae		125	7.6
Ostreidae		99	6.0
Limidae		30	1.8
Galeommatidae		9	0.6
Arcidae		3	0.2
Veneridae		3	0.2
Ungulidae		2	0.1
Carditidae		1	0.1
Isognomonidae		1	0.1
Plicatulidae		1	0.1
Mytilidae	<i>Musculista glaberrima</i> (Dunker)	559	34.1
Pectinidae	<i>Chlamys curtisiana</i> (Iredale)	196	12.0
Pteriidae	<i>Pteria</i> sp.	137	8.4
Pteriidae	<i>Pteria breviaolata</i> (Dunker)*	115	7.0
Pinnidae	<i>Pinna bicolor</i> Gmelin*	110	6.7
Pectinidae	<i>Electroma</i> sp.	92	5.6
Malleidae	<i>Malleus atrinus</i> Gmelin	74	4.5
Ostreidae	<i>Dendostrea folium</i> (Linnaeus)	60	3.7
Malleidae	<i>Malleus albus</i> Lamarck	41	2.5
Pteriidae	<i>Pinctada maxima</i> (Jameson)*	39	2.4
Pteriidae	<i>Pinctada albina</i> (Lamarck)*	34	2.1
Pteriidae	<i>Pteria penguin</i> (Roding)*	31	1.9
Ostreidae	<i>Saccosiraea cucullata</i> Born*	29	1.8
Limidae	<i>Limatula</i> sp.	23	1.4
Malleidae	<i>Malleus daemonicus</i> Reeve	12	0.7
Pectinidae	<i>Mimachlamys gloriosa</i> (Reeve)	10	0.6
Pectinidae	<i>Complicachlamys dringi</i> (Reeve)	9	0.6
Ostreidae	<i>Ostrea</i> sp.	9	0.6
Galeommatidae	<i>Scintilla</i> sp.	9	0.6
Pinnidae	<i>Atrina pectinata</i> (Linnaeus)	7	0.4
Pinnidae	<i>Pinna incurva</i> Gmelin	7	0.4
Pteriidae	<i>Electroma zebra</i> Reeve	4	0.2
Limidae	<i>Limaria fragilis</i> (Gmelin)	3	0.2
Pectinidae	<i>Chlamys squamata</i> (Gmelin)	2	0.1
Ungulidae	<i>Diplodonta</i> sp.	2	0.1
Limidae	<i>Lima lima vulgaris</i> Linnaeus	2	0.1
Mytilidae	<i>Musculista cumingiana</i> (Reeve)	2	0.1
Pteriidae	<i>Pinctada margaritifera</i> Linnaeus	2	0.1
Arcidae	<i>Acar</i> sp.	1	0.1
Veneridae	<i>Antigona lamellaris</i> Schumacher	1	0.1
Arcidae	<i>Arca avellana</i> Lamarck	1	0.1
Arcidae	<i>Arca navicularis</i> Brugiere	1	0.1
Carditidae	<i>Cardita cardioides</i> (Reeve)	1	0.1
Pectinidae	<i>Decatopecten strangei</i> (Reeve)	1	0.1
Isognomonidae	<i>Isognomon isognomon</i> (Linnaeus)	1	0.1
Limidae	<i>Lima</i> sp.	1	0.1
Limidae	<i>Limatula tadena</i> (Iredale)	1	0.1
Malleidae	<i>Malleus malleus</i> Linnaeus	1	0.1
Pectinidae	<i>Mimachlamys deliciosa</i> (Iredale)	1	0.1
Veneridae	<i>Paphia gallus</i> (Gmelin)	1	0.1
Pteriidae	<i>Pinctada</i> sp.	1	0.1
Pinnidae	<i>Pinna deltodes</i> Menke	1	0.1
Plicatulidae	<i>Plicatula essingtonensis</i> Iredale	1	0.1

TABLE 2.

continued

Family	Species	Frequency	
		No.	%
Pteriidae	<i>Pteria tortirostris</i> (Dunker)	1	0.1
Ostreidae	<i>Saccostraea echinatum</i> Qouy and Gaimard	1	0.1
Veneridae	<i>Tapes sulcarius</i> (Lamarck)	1	0.1
Malleidae	<i>Vulsella vulsella</i> Linnaeus	1	0.1

\* Denotes species for which growth rates and length frequency histograms are given.

The frequency of occurrence by number and percentage is given for each family and species.

higher settlement during the warmer months of the wet season (October to March), and significantly higher settlement rates January–March than March–June (Scheffe's  $P < 0.05$ ). This trend was also apparent in individual species (*P. bicolor*, *Malleus albus* and *P. penguin*), and was significant (Scheffe's  $P < 0.05$ ) for *P. maxima*, *Electroma* sp., *M. atrinus*, *Musculista glaberrima* and *Pteria* sp. (Table 3).

Length frequencies of potential commercial species together with their mean growth rate for each period are shown in Figure 3a–f. The size range of *P. maxima* found on collectors was between 3 mm and 16 mm with an average of 8.3 mm. This corresponded to an average growth rate of  $5.8 \text{ mm} \cdot \text{month}^{-1}$ . A similar growth rate was evident for *P. albina* ( $5.2 \text{ mm} \cdot \text{month}^{-1}$ ) and *S. cucullata* ( $6.6 \text{ mm} \cdot \text{month}^{-1}$ ). Fan shells (Pinnidae) had the fastest growth rates, with *P. bicolor* averaging  $20.7 \text{ mm} \cdot \text{month}^{-1}$  throughout the year. Unlike other species, there was no apparent single peak in length frequencies of *P. bicolor*, with lengths during any period having a very wide range (Fig. 3c).

## DISCUSSION

This study demonstrates that a wide range of tropical bivalve species settle on artificial collectors. A relatively small number of species accounted for most of the bivalves caught and some of these have commercial value, especially *P. maxima*.

Of the bivalve families found on the collectors, pearl oysters (Pteriidae), which were the second most abundant family, have the greatest commercial significance. This is mainly because of their ability to produce pearls, but also for their lustrous nacre and edible meat. *Pteria* sp., *P. breviaolata*, *P. penguin* and *P. albina* were common, but their commercial value is not as high as *P. maxima* and *P. margaritifera* which produce the most valuable cultured pearls.

Australia's pearl culture industry is worth over A\$130 million annually and largely depends on wildstock fisheries for *P. maxima* to supply its culture stocks (McLoughlin et al. 1994). Divers collect oysters, and because most commercial pearl oyster beds off the Northern Territory are relatively deep (>30 m), costs of collection and transportation are high, A\$17–27 per oyster (Rose and Baker 1994). With such high costs, there are obvious benefits in developing other methods to collect *P. maxima*. Culture of spat in hatcheries is proving successful (Rose and Baker 1994), but use of artificial collectors in the natural environment may provide a cheaper alternative with the advantage that, being from a broader genetic pool, the spat may have already undergone significant

TABLE 3.

Settlement rates (No. of individuals  $\cdot$  30 g bag<sup>-1</sup>  $\cdot$  month<sup>-1</sup>  $\pm$  standard deviation) of common species of bivalves collected on artificial collectors set in the Timor Sea.

Species	Settlement Rate					
	Jun–Aug (9)	Aug–Oct (27)	Oct–Jan (15)	Jan–Mar (19)	Mar–Jun (16)	Total (86)
<i>Musculista glaberrima</i>	0.9 $\pm$ 1.5	2.3 $\pm$ 2.8*	6.0 $\pm$ 7.2*	3.5 $\pm$ 3.4*	0.6 $\pm$ 0.7	2.7 $\pm$ 4.1
<i>Chlamys curtisiana</i>	2.5 $\pm$ 3.0*	1.1 $\pm$ 1.7	0.9 $\pm$ 1.0	0.8 $\pm$ 1.1	0.3 $\pm$ 0.8	1.0 $\pm$ 1.6
<i>Pteria</i> sp.	0	0.0 $\pm$ 0.1	0.7 $\pm$ 1.0	2.5 $\pm$ 4.1*	0.2 $\pm$ 0.5	0.7 $\pm$ 2.2
<i>Pteria brevilata</i>	0.6 $\pm$ 0.5	0.2 $\pm$ 0.4	0.5 $\pm$ 0.6	1.3 $\pm$ 3.1	0.4 $\pm$ 0.5	0.6 $\pm$ 1.6
<i>Pinna bicolor</i>	0.2 $\pm$ 0.3	0.5 $\pm$ 0.6	0.7 $\pm$ 0.9	0.6 $\pm$ 1.1	0.1 $\pm$ 0.2	0.5 $\pm$ 0.7
<i>Electroma</i> sp.	0.1 $\pm$ 0.2	0.2 $\pm$ 0.4	0	2.2 $\pm$ 3.1*	0	0.5 $\pm$ 1.7
<i>Malleus atrinus</i>	0	0.1 $\pm$ 0.6	1.1 $\pm$ 1.9*	0.3 $\pm$ 0.4	0.1 $\pm$ 0.2	0.3 $\pm$ 1.0
<i>Dendostraea folium</i>	0.4 $\pm$ 0.6	0.4 $\pm$ 0.7	0.3 $\pm$ 0.4	0.2 $\pm$ 0.7	0.1 $\pm$ 0.2	0.3 $\pm$ 0.6
<i>Malleus albus</i>	0.1 $\pm$ 0.2	0.1 $\pm$ 0.4	0.3 $\pm$ 0.5	0.3 $\pm$ 0.6	0.1 $\pm$ 0.2	0.2 $\pm$ 0.4
<i>Pinctada maxima</i>	0	0.1 $\pm$ 0.3	0.6 $\pm$ 0.6*	0.0 $\pm$ 0.1	0.2 $\pm$ 0.3	0.2 $\pm$ 0.4
<i>Pinctada albina</i>	0.1 $\pm$ 0.2	0.4 $\pm$ 0.7	0.2 $\pm$ 0.4	0.0 $\pm$ 0.1	0.1 $\pm$ 0.3	0.2 $\pm$ 0.5
<i>Pteria penguin</i>	0	0	0.4 $\pm$ 1.0	0.2 $\pm$ 0.4	0.1 $\pm$ 0.2	0.1 $\pm$ 0.5
<i>Saccostraea cucullata</i>	0	0.2 $\pm$ 0.7	0.1 $\pm$ 0.1	0.0 $\pm$ 0.1	0.3 $\pm$ 0.4	0.1 $\pm$ 0.5

Sample numbers are in parentheses and periods which had significantly higher (Scheffe's  $P > 0.05$ ) settlement rates than other periods are indicated (\*).

selective pressures, possibly making them more resistant to disease and infection during growout.

In the present study, *P. maxima* represented 2.4% of the total number of bivalves collected, with settlement rates during October to January at an average of 0.6 individuals  $\cdot$  30 g bag<sup>-1</sup>  $\cdot$  month<sup>-1</sup>. This settlement rate appears low, but it is difficult to determine what settlement rates would be required to make the collection technique viable. The present study used a number of small collectors ( $\approx$ 30 g monofilament) to act as replicates for experimental design requirements and total numbers were consequently low. If larger collectors were used, specifically designed to collect large numbers of bivalves, they might be a viable collection technique in waters off northern Australia. A major improvement to the design of the collectors used in this study would have been to provide a rigid mesh frame around the bags as done by Scoones (1991), to protect them from the significant damage presumably caused by fish and turtles.

Apart from the possibility of using artificial collectors to collect pearl oysters for aquaculture, they also have potential as a research tool. Pearl oyster fisheries target small oysters (120–200 mm), because they are more appropriate for pearl culture (Knuckey, unpubl. data). However, similar to most molluscan fisheries, highly variable settlement and recruitment patterns are typical in pearl oyster fisheries (McLoughlin et al. 1994) and can lead to patchy distribution and abundance. This appears especially true in the Northern Territory, where many of the fishable pearl oyster beds are on isolated shoals. Use of artificial collectors to measure relative settlement rates on different shoals could help to predict potential fishing grounds, thereby reducing search time and increasing the economic viability of fishing a patchy resource. In the scallop fishery in southern Australia, the relative numbers of larvae that settle on spat collectors can be related to the size of the parental population (Young et al. 1988) and to recruitment to the fishery during the next year (Sause et al. 1987). In this manner, collectors can be a useful stock assessment tool to assist fishery management, and could be equally well applied to stock assessment and management of pearl oyster fisheries. It is important to

note however, that settlement of bivalves on collectors only indicates the presence of larvae competent to settle, and will not necessarily reflect abundances of bivalves on the natural substrate (Young et al. 1988), which can be influenced by numerous physical and biological factors.

*P. maxima* had highest settlement rates during October to January, with lesser rates in other months, and none from June to August, which agrees with other work in northern Australia (Wada 1953), which showed *P. maxima* usually begins to spawn in late October and continues for several months, with a small number of animals spawning throughout the year. Bwathondi and Ngoile (1982) also found settlement of *Pinctada* spp. was highest during the wet season (November and December). Other bivalves (e.g., *P. bicolor*, *Electroma* sp., *M. atrinus*, *M. glaberrima*, *Pteria* sp.) showed higher settlement rates during the wet season, although this was not always significant, possibly due to extremely high variability in settlement rates. At this time of year, north-westerly monsoons cause high rainfalls in coastal northern Australia, and increased productivity caused by warmer water temperatures and the large amounts of nutrients washed into the ocean might be advantageous to settlement during this time. Dayton et al. (1989) recorded massive numbers of almost identically sized *P. maxima* spat on coral collectors set in the Great Barrier Reef between October and February, which suggests they settled almost instantaneously. Similarly, only one settlement peak of *P. maxima* was apparent in any one period during this study, but sizes ranged between 3 and 16 mm. Whether this pattern resulted from an instantaneous settlement event with variable growth rates, or continuous settlement over numerous weeks, could not be determined in this study. Results did indicate there was more than one settlement event throughout the year, and can support the findings of Rose et al. (1990) that there is a main spawning period in September/October and a secondary spawning in March/April.

Growth rates of individual *P. maxima* in this study reached 10.4 mm  $\cdot$  month<sup>-1</sup> with an average of 5.8 mm  $\cdot$  month<sup>-1</sup>, slightly lower than those observed by Rose and Baker (1994) of 7–9 mm  $\cdot$  month<sup>-1</sup> for spat cultured in plastic trays in the sea.

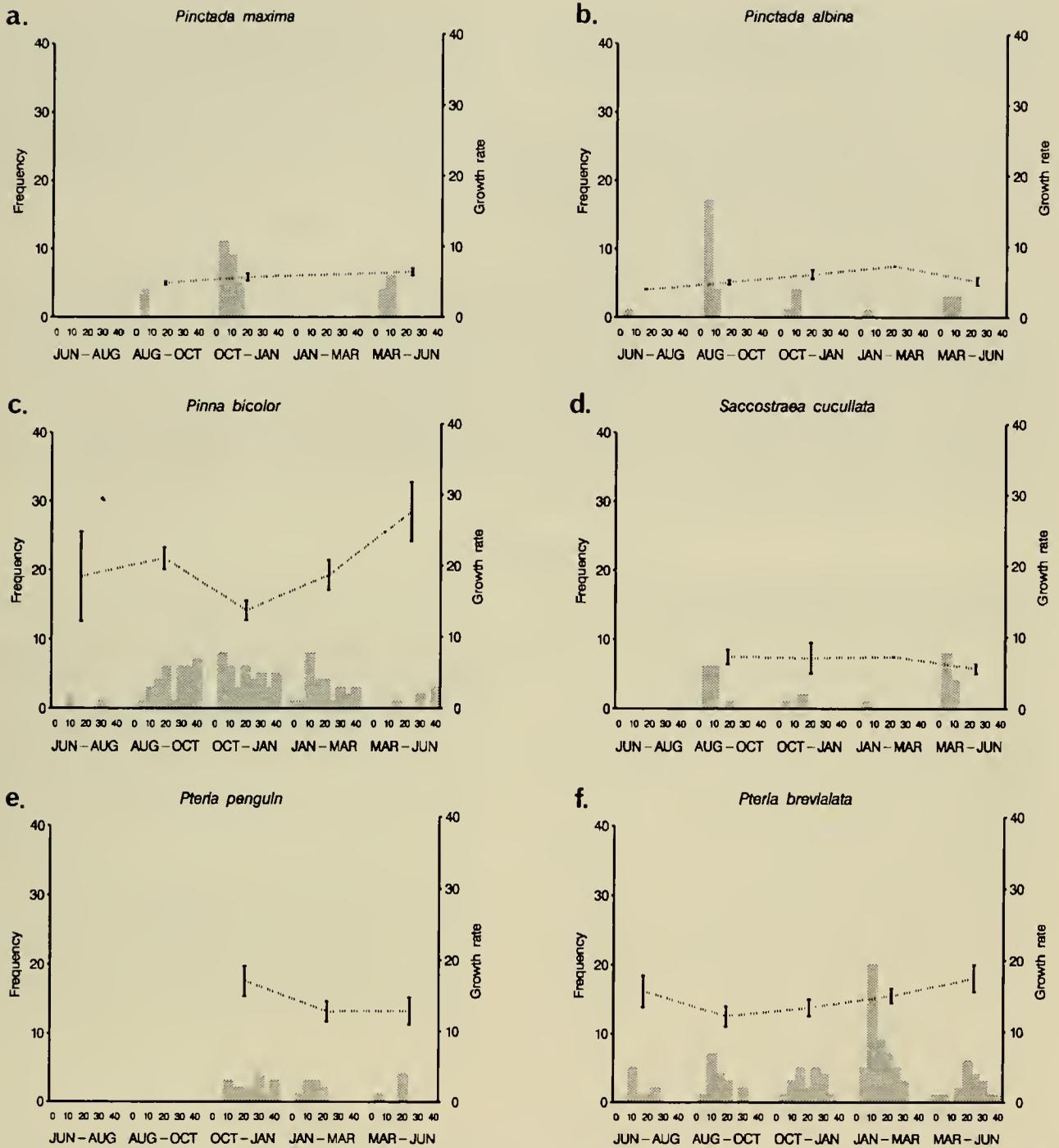


Figure 3. Length frequencies and growth rates of bivalve species with commercial potential caught on collectors over 5 periods during one year in the Timor Sea. Dotted lines connect error bars of mean estimated growth rates ( $\text{mm} \cdot \text{month}^{-1} \text{DVL}$ )  $\pm$  standard error. Shaded histograms represent the length frequencies of each species in 5 mm size classes from 0–40 mm.

Importantly, growth rates in this study could be overestimated by the assumption that the spat settled mid-way during the period, because spat that settled near the beginning of the period would grow and could be identified, whereas spat that settled near the end of the period would possibly be undetected. Nonetheless, it would be reasonable that growth of *P. maxima* was less in collectors, where there is close competition for space and food with numerous

other fauna, than in plastic trays. Rose and Baker (1994) noted reduced growth rate in spat stocked at increased density.

In contrast to seasonal effects, bivalve settlement rates were rarely different over the spatial scales used in this study (1 m–15 km). Wide variation in the settlement rates on individual collectors possibly masked most spatial effects. Such variation may have resulted from aggregated settlement, such as noted in *P. maxima*

(Rose and Baker 1994) which may lead to an all-or-none scenario on the collectors. Alternatively, it may be that the patchiness of larval settlement occurs on a very small scale (<1 m) even though the collectors were in open water and subject to significant tidal currents over long periods.

Although the two most common species that settled on the collectors (*M. glaberrima* and *Chlamys curtisiana*) have little value, some bivalves besides the Pteriidae do have commercial potential. Fan shells (Pinnidae) are large shells, common throughout the Indo-West Pacific region, and although they may produce pearls of moderate value, they are mostly valued for human consumption (Wells and Bryce 1988) or as bait for catching fish (Butler and Brewster 1979). They were common in the present study; *P. bicolor* had high settlement rates throughout the year and *A. pectinata*, *P. incurva*, and *P. deltodes* were also found. *P. bicolor* can reach lengths of up to 400 mm (Butler and Brewster 1979) and the fast growth rate and common occurrence exhibited in the present study, suggest this species and method of capture may have commercial potential for culture or harvest. Edible oysters (*S. cucullata*, *S. echinatum* and *Ostrea* sp.) were also found on the collectors and, like more temperate species of the Ostreidae

(e.g. *Ostrea edulis* Linnaeus and *O. angasi* Sowerby), are highly valued for their taste and have considerable potential for culture.

Artificial collectors deployed in the natural environment were successful in collecting tropical bivalve species of commercial importance. Whether such collectors are used for aquaculture depends on their ability to collect commercially viable numbers, but they could have important uses as a stock assessment tool for managing the *P. maxima* fishery.

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## HOST-PARASITE RELATIONSHIPS BETWEEN THE MUSSEL, *MYTILUS EDULIS* L., AND THE PEA CRAB, *TUMIDOTHERES MACULATUS* (SAY), IN THE SOUTHWESTERN ATLANTIC

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**ABSTRACT** Incidence and effects of the pea crab *Tumidotheres maculatus* (Say) on a population of the mussel, *Mytilus edulis* L., were studied at an offshore bank near Quequén, Argentina (38°50'S, 58°55'W). Seven samples were obtained randomly, April to October 1990, among benthic invertebrates usually discarded by the benthic-demersal fishery. Infestation by pea crabs varied from 54.3 to 72.6% (average: 63.6%), which is considerably higher than the incidence recorded at the same bank in 1970. The null hypothesis of random pea crab distribution among mussels was tested by fitting the data to a Poisson distribution. Male crabs were randomly distributed in their hosts. Significantly more Stage IV-V female crabs were single occupants of a mussel than expected. On the contrary, pre-hard crabs had a clumped distribution in their hosts. Correlation between mussel length and pea crab size was low but significant for Stage IV-V female crabs, absent for male crabs. Mussel length was significantly lower when parasitized by mature females ( $P < 0.001$ ) but not when harboring other pea crab stages ( $P > 0.05$ ). Mussels of a given length had significantly lower meat dry weight when parasitized by Stage IV-V female crabs ( $P < 0.01$ ). Weight differences between mussels harboring other pea crab stages and uninfested mussels were not significant ( $P > 0.05$ ). Our results confirm adverse effects of female *T. maculatus* on *M. edulis*.

**KEY WORDS:** Pea crabs, mussels, *Tumidotheres maculatus*, *Mytilus edulis*, Argentina

### INTRODUCTION

Although formerly considered to be commensals, there is increasing evidence that some species of pinnotherid crabs produce adverse effects on hosts. Several studies have reported that pea crabs cause damage or malformations to gills, gonads, and other bivalve tissues (Stauber 1945, Christensen and McDermott 1958, Haven 1958, Dix 1973, Jones 1977), and also decrease filtration and oxygen consumption rates (Bierbaum and Shumway 1988), as well as size and weight of their hosts (Haven 1958, Seed 1969, Kruczynski 1972, Anderson 1975, Pregenzer 1981, Bierbaum and Ferson 1986).

Presence of pea crabs within the mantle cavities of the Argentine and Uruguayan mussel is well known (Rathbun 1933, Barattini and Ureta 1960, Castellanos 1962, Szidat 1965, Williams 1965, Ringuet 1967). The geographic distribution and hosts of *Tumidotheres maculatus* (Say) (= *Pinnotheres maculatus*, see Campos 1989) in Argentina have been studied by Fenucci (1975) and Gómez-Simes (1993). Fenucci (1971, 1973) has reported ecological information, particularly on percentages of infested mussels on offshore banks near Mar del Plata and Quequén (Argentina).

*T. maculatus* females spend most of their adult lives within host mussels, which they are unable to leave because the mussel's gape is not large enough. On the other hand, males are smaller and are able to move freely from host to host (Bierbaum and Ferson 1986). Larval stages of *T. maculatus* have been described by Costlow and Bookhout (1966). Reproduction and feeding habits of this species have been dealt with by Pearce (1964) and Caine (1975), respectively.

The purposes of this study were to: (1) calculate the incidence of *T. maculatus* in an offshore population of *Mytilus edulis* L. near Quequén and compare it with previous information for this area; (2) analyze the co-occurrence of pea crabs in their hosts; and (3)

compare the length and weight of mussels harboring mature female crabs or other pea crab stages with those of uninfested mussels.

### MATERIALS

The mussel exploited from banks off Buenos Aires Province has been referred to as *Mytilus platensis* d'Orbigny (Castellanos 1962, Penchaszadeh 1979) or *Mytilus edulis platensis* (Lasta et al. 1986). Recent studies using multivariate analysis of morphometric and allozyme electrophoresis data (McDonald et al. 1991, Seed 1992) have concluded that both *M. platensis* and the Chilean and Patagonian mussel, *Mytilus chilensis* Hupé, are conspecific with the North Atlantic mussel, *M. edulis*. Although Argentine mussels may be very close to the European blue mussel from the genetic and morphological points of view, typically they occur in extensive banks on the continental shelf. They are found in intertidal (Penchaszadeh 1973) and subtidal (Amaro 1979) areas less frequently.

Offshore *M. edulis* banks occur in a patchy distribution between depths of 36 and 58 m, off Buenos Aires Province, Argentina, and Uruguay (Penchaszadeh 1979). Faro Querandí and Quequén banks were heavily exploited during the 1960s and 1970s. At the beginning of the 1980s, the former was already abandoned due to low yields, and the latter was producing remarkably low captures (Lasta et al. 1986). During the present study there was no commercial exploitation in the Quequén Bank.

Seven samples were obtained April to October 1990, at approximately monthly intervals (Table 1), in an area located ca 30 km SW of Quequén Harbor (38°50'S, 58°55'W), at a depth of ca 44 m (Fig. 1). This area is known as "Necochea Bank" (Penchaszadeh 1979) or "Quequén Bank" (Lasta et al. 1986). Mussels were obtained incidentally by the coastal fishing boat *Felisitas* during capture of benthic-demersal fishes. They were randomly sampled among benthic invertebrates usually discarded by the fishermen. Sampling was interrupted in November 1990 because the coastal fleet at Quequén Harbor shifted to the more profitable soupfin shark (*Galeorhinus galeus* Blainville) midwater fishery.

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TABLE 1.

Number of *M. edulis* in each sample, percentage of infestation, and length (mean  $\pm$  standard deviation) of mussels without pea crabs, with stage IV-V females, and with other stages (pre-hard stages, males, and stage I-III females) of *T. maculatus*.

Date	No.	% Infested	Length (mm)		
			Without	IV-V Females	Other Stages
25/04/90	249	63.5	57.6 $\pm$ 5.5	52.8 $\pm$ 5.5	57.3 $\pm$ 5.1
25/05/90	88	71.6	62.5 $\pm$ 6.7	57.7 $\pm$ 6.1	63.6 $\pm$ 4.6
25/06/90	51	56.9	64.6 $\pm$ 5.2	59.0 $\pm$ 4.5	61.4 $\pm$ 5.8
30/07/90	95	72.6	65.0 $\pm$ 5.9	59.3 $\pm$ 6.3	63.7 $\pm$ 4.6
25/08/90	89	62.9	61.4 $\pm$ 6.8	59.4 $\pm$ 5.4	61.7 $\pm$ 6.2
04/09/90	153	54.3	62.3 $\pm$ 7.3	59.6 $\pm$ 5.9	62.9 $\pm$ 7.0
09/10/90	125	66.4	63.6 $\pm$ 6.8	58.8 $\pm$ 5.8	65.9 $\pm$ 7.4
TOTAL	850	63.6	61.4 $\pm$ 6.9	57.8 $\pm$ 6.2	60.9 $\pm$ 6.5

The developmental cycle of pea-crabs shows similar features in several species of this family (Atkins 1926, Christensen and McDermott 1958, Pearce 1966a,b). Pearce (1964) has summarized the life cycle of *T. maculatus* as follows: after the planktonic larval stages, the megalops molt into a first crab which becomes associated with a host. The following instars are small, soft-shelled, and are known as pre-hard stages. Then, both sexes molt into a well-calcified, highly pigmented hard stage (Stage I), which leave their hosts to copulate in open water. The external morphology of both sexes is very similar throughout the pre-hard series and the Stage I instar. Following copulation, females again infest a host, and molt into a soft post-hard instar (Stage II). It is followed by Stages III, IV and V characterized by an overall increase in size. The Stage V crab is the terminal adult female.

As Stage IV-V females are remarkably larger than the previous post-hard or hard stages, we analyzed separately the effect of these 2 stages on the length and weight of *M. edulis*.

#### Incidence of *T. maculatus*

Samples were fixed in neutralized 5% formalin. Mussels were opened and their mantle cavities carefully inspected for *T. maculatus* specimens, which were transferred to 70% ethanol for preservation. Mussel shell length was measured with calipers to the nearest 0.1 mm. Pea crab carapace width was measured to the nearest 0.1 mm with a micrometer eyepiece under a binocular microscope.

The percentage of *M. edulis* containing *T. maculatus* varied from 54.3 to 72.6% (Table 1), averaging 63.6% for all mussels. A high percentage of bivalves harboring only pre-hard crabs was recorded in April (Fig. 2). Szidat (1965), the first author who studied pea crab occurrence in banks off Buenos Aires Province, found that 4-6% of the mussels were infested by pea crabs. Samplings carried out on mussels obtained by the local fishery, when the banks were under heavy exploitation, found that infestation rates varied from 7.0 to 16.5% (mean: 11.6%) between April and September 1970 in Quequén Bank, and from 18.2 to 32.0% (mean: 25.4%) between February and October 1970 in Faro Querandí Bank (Fenucci 1971). A comprehensive sampling carried out in November 1971 in Faro Querandí Bank found that 20.1% of mussels contained *T. maculatus* (Fenucci 1973). A sample from the continental shelf off Uruguay in 1977 had an infestation rate of 13.6% (Amaro 1979).

Pea crabs are absent in intertidal and subtidal banks along the Uruguayan coast (Amaro 1979). Kruczynski (1974) has reported that infestation rates of *T. maculatus* in *M. edulis* were higher in subtidal than in intertidal areas near Woods Hole, MA.

Present results (mean: 63.6%) show that *T. maculatus* incidence has increased remarkably in offshore *M. edulis* populations since Fenucci's (1971) study 20 years ago. Both studies were carried out in the same area and during the same seasons (autumn-winter). The causes of this phenomenon are not clear, but could be related to the interruption of the mussel fishery in the last years. Kruczynski (1973) suggested that low infestation rates in the scallop *Argopecten irradians concentricus* (Say) were also related to commercial exploitation.

Female pea crabs outnumbered males over the period of study. This was expected, since an unknown proportion of Stage I males



Figure 1. Geographic location of Quequén Bank.

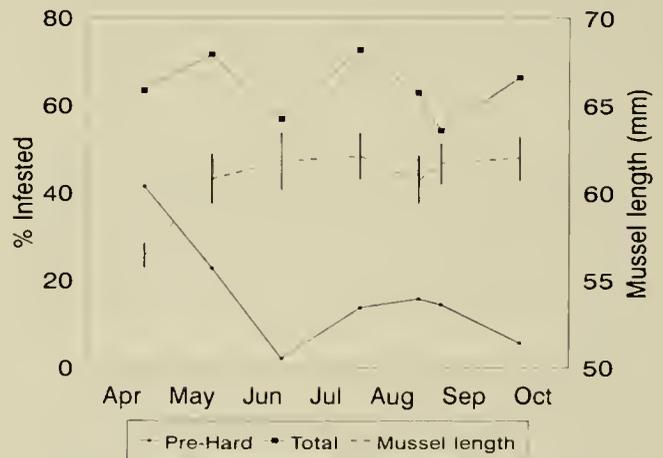


Figure 2. Percentage of mussels infested by pea crabs (total or only pre-hard stages), and mean mussel length ( $\pm$ 95% confidence interval) over the study period.

may be found outside mussels. Pre-hard stages occurred from April to October, with a maximum in April (Table 2).

Present results clearly show that infestation rate decreases with increasing mussel length. Maximum infestation (80%) was recorded in 48–52 mm mussels, i.e., having a size 12 mm smaller than the most abundant size class. Incidence of *T. maculatus* decreased to 40% with increasing length (Fig. 3). Fenucci (1973) found that the infestation rate was 21–30% in mussels from 45 to 80 mm. Infestation increased with greater mussel length, but the number of observations were very low. Seed (1969) reported a positive relationship between infestation rate and host size. He suggested that percentage of crabs could be directly proportional to the amount of filtered water or to the time that mussels had been exposed to infestation. Decrease of infestation with increasing host size might be related to pea crab mortality.

More than 50% of the mussels below 64 mm were infested by mature females. On the other hand, a small percentage of mussels larger than 64 mm harbored Stage IV–V females. Infestation by pre-hard stages showed similar values along a wide range of mussel sizes (Fig. 3). On the contrary, Pearce (1966a) has found the highest percentage of *Fabia subquadrata* Dana first crab stages in the smallest host mussels, *Modiolus modiolus* (L.). In the sample showing maximum recruitment (April), mussels harboring pre-hard stages had the same mean length as uninfested mussels (mean length: 57.6 mm in both groups, ANOVA,  $P > 0.05$ ). This can be regarded as an evidence suggesting that in this case, the invasive stage of *T. maculatus* does not select a particular host size. In this study, *M. edulis* was invaded by *T. maculatus* at a length of ca. 40 mm, although the smaller infested mussel measured 31.5 mm. Minimum size of *M. edulis* that can be occupied by *Pinnotheres pisum* (Pennant) was suggested by Seed (1969) as 35 mm.

*Co-Occurrence of Crabs in Mussels*

Frequency of mussels harboring zero, one, or multiple pre-hard, male, or Stage IV–V female pea crabs was compared with a Poisson model, to test the null hypothesis that crab number per mussel was due to chance. If this hypothesis is rejected (variance/mean ratio significantly different from unity), it can be concluded that pea crab distribution in their hosts is clumped or uniform. The goodness of fit of the Poisson distribution was assessed using standard  $\chi^2$  tests (Sokal and Rohlf 1981). Expected frequencies less than 3 were merged.

Male pea crabs were randomly distributed in their hosts ( $\chi^2$ : 1.82,  $P > 0.05$ , variance/mean ratio: 1.06) (Fig. 4). The analysis

TABLE 2.

Total number and percentage of each category of *T. maculatus* in *M. edulis*, Quequén Bank.

Date	No.	% Pre-Hard Stages	% Males	% St. I-III Females	% St. IV-V Females
25/04/90	234	72.2	1.7	2.2	23.9
25/05/90	73	34.3	12.3	2.7	50.7
25/06/90	31	6.4	16.1	6.5	71.0
30/07/90	82	23.2	17.1	8.5	51.2
25/08/90	58	24.1	12.1	6.9	56.9
04/09/90	87	26.4	18.4	4.6	50.6
09/10/90	85	8.2	18.8	7.1	65.9
TOTAL	650	39.9	10.9	4.6	44.6

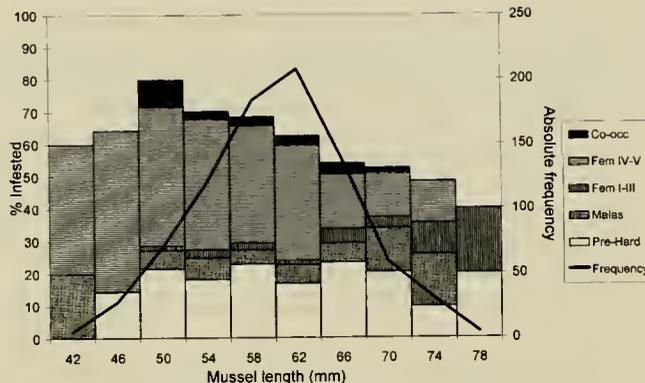


Figure 3. The histogram shows the relationship between the length of mussel host and incidence of pre-hard stages, males, Stage I–III and Stage IV–V females, and co-occurrence of different stages of *T. maculatus*. The size frequency distribution of mussels is shown by a line. Two specimens (31.5 mm, harboring a female, and 39.5 mm, uninfested) were omitted.

of Stage IV–V female number per mussel indicated that the observed cases differed significantly from the expected frequencies according to a Poisson distribution ( $\chi^2$ : 89.2,  $P < 0.001$ , variance/mean ratio: 0.64) (Fig. 4). More Stage IV–V females were single occupants of a host than expected by a random distribution. As a consequence, there were fewer multiple occupants or uninfested hosts than expected. On the contrary, pre-hard *T. maculatus* showed a highly significant clumped distribution ( $\chi^2$ : 94.2,  $P < 0.001$ , variance/mean ratio: 1.60) (Fig. 4). Fewer single occupants, and more multiple occupants or empty hosts were observed, than expected for a Poisson distribution.

In addition, co-occurrence of pre-hard, male, and Stage IV–V female pea crabs in the same mussel host was analyzed using the G statistic in two-way contingency table tests (Sokal and Rohlf 1981). The expected frequency of each cell was calculated under

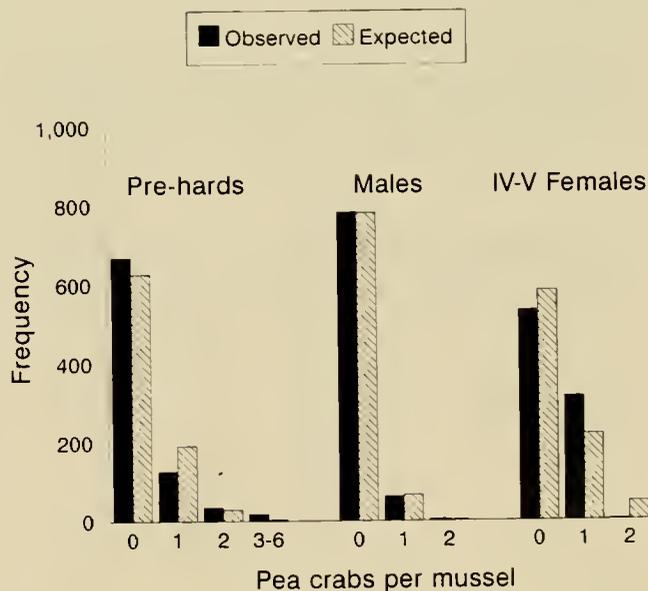


Figure 4. Number of observed pre-hard, male and Stage IV–V female pea crabs in each host and expected Poisson frequencies.  $\chi^2$  tests: pre-hard stages,  $\chi^2 = 94.2$  ( $P < 0.001$ ); males,  $\chi^2 = 1.82$  ( $P > 0.05$ ); Stage IV–V females,  $\chi^2 = 89.2$  ( $P < 0.001$ ).

the null hypothesis that occurrences of different sexes or stages were independent events. Observed number of pre-hard, male, or Stage IV-V female pea crab co-occurrences were significantly lower than those expected under the null hypothesis (Table 3).

#### Pea Crab Infestation and Mussel Length

The correlation between carapace width of *T. maculatus* Stage IV-V females and length of the mussels harboring them was low, but highly significant ( $r = 0.311$ ;  $n = 302$ ;  $P < 0.001$ ). On the contrary, width of male crabs was uncorrelated with mussel length ( $r = 0.029$ ;  $n = 73$ ;  $P > 0.05$ ). A positive correlation between female *P. pisum* carapace width and *M. edulis* length has also been reported (Seed 1969, Haines et al. 1994). Fenucci (1973), however, did not find a correlation between female *T. maculatus* size and host length.

The effect of time and pea crab infestation on mussel length was analyzed by a two-way analysis of variance (ANOVA), using the SYSTAT statistical package (Wilkinson 1990). Factors were time (7 samples) and pea crab occurrence (absence, presence of Stage IV-V females, presence of other stages: pre-hard stages, males and Stage I-III females). Using a two-way ANOVA we were able to separate mussel length variation through time from the error mean square. Variation in mussel length through time was expected, and may be caused by growth or size variations among patches within the mussel bed, but its analysis was not the purpose of this study. Normality and variance homogeneity were verified in all samples using Kolmogorov-Smirnov's and Bartlett's tests, respectively (Sokal and Rohlf 1981). Two *post-hoc* contrasts were carried out: (1) mussels with Stage IV-V female crabs vs. those without crabs; and (2) mussels with all other stages vs. those without crabs. Mussels harboring different combinations of Stage IV-V female and male, or female and pre-hard crabs, were classified as harboring Stage IV-V females, since previous studies (Kruczynski 1972, Pregonzer 1981) indicated that the latter have a stronger influence on shell size than either males or pre-hard stages.

The ANOVA of *M. edulis* length data (Table 4) showed that there were highly significant differences due to time and pea crab occurrence ( $P < 0.001$ ), whereas the interaction between both factors was non-significant ( $P > 0.05$ ). As can be seen in Figure 2, the small mussels collected in April were responsible for the

TABLE 3.

Two-way contingency tables showing observed cases of infestation by pre-hard, male, and Stage IV-V female pea crabs in *M. edulis*.

	Without Males	With Males	
Without IV-V Females	474 (491)	59 (42)	Gw = 23.2
With IV-V Females	309 (292)	8 (25)	P < 0.001
	Without Pre-Hards	With Pre-Hards	
Without IV-V Females	365 (420)	168 (113)	Gw = 110.9
With IV-V Females	305 (250)	12 (67)	P < 0.001
Without Males	606 (617)	177 (166)	Gw = 16.0
With Males	64 (53)	3 (14)	P < 0.001

Expected frequencies under the null hypothesis of independence appear in brackets. Gw: G statistic using William's correction.

TABLE 4.

Two-way analysis of variance of mussel length. Factors are time (7 dates) and infestation: without pea crabs, with stage IV-V females, and with other stages (pre-hard stages, males and Stage I-III females).

Source	SS	DF	MS	F	P
Time	6130.7	6	1021.8	28.43	<0.001
Infestation	2952.5	2	1476.3	41.07	<0.001
IV-V Females vs. Without	2376.6	1	2376.6	66.12	<0.001
Other Stages vs. Without	0.3	1	0.3	0.01	0.922
Time × Infestation	467.9	12	39.0	1.09	0.369
Error	29798.7	829	35.9		

SS: sum of squares, DF: degrees of freedom, MS: mean square.

significant differences in the time factor. Mussels harboring Stage IV-V female crabs had significantly lower lengths (Table 1; Fig. 3) than uninfested mussels ( $P < 0.001$ ). On the other hand, differences between mussels containing other stages and uninfested mussels were non-significant ( $P > 0.05$ ).

This field study agrees with previous experiments demonstrating adverse effects of *T. maculatus* on mussels and scallops (Kruczynski 1972, Bierbaum and Ferson 1986, Bierbaum and Shumway 1988). Our results indicate that only Stage IV-V females are associated with mussels showing decreased length in banks off the Argentine coast. This fact must be taken into account in future studies on size-age relationship in mussels, since individuals harboring mature female pea crabs should be discarded from such analyses.

#### Pea Crab Infestation and Mussel Weight

Mussel soft tissues from the most abundant samples (April and September) were oven-dried at 60°C for 72 hr and weighed on an analytical balance to the nearest mg.

Regression of mussel dry weight on shell length using the SYSTAT package (Wilkinson 1990), was carried out to compare dry weight of mussels harboring Stage IV-V females or other pea crab stages with that of uninfested mussels. In addition, analysis of covariance (ANCOVA), with mussel length introduced as a covariate, was used to test for significant dry weight differences due to pea crab occurrence. Lengths and weights were ln-transformed to linearize the exponential relationship between these variables, and also to satisfy the assumptions of the ANCOVA model.

Results of regression analysis can be seen in Figure 5. The analysis of mussel dry weight with ANCOVA (Table 5) showed that, for a given length, hosts harboring Stage IV-V female pea crabs had significantly less biomass than uninfested hosts (April:  $P < 0.001$ ; September:  $P < 0.01$ ). On the contrary, differences between mussels harboring other pea crab stages and mussels without crabs were non-significant ( $P > 0.05$  in both months). Interactions between treatments and the covariate were always non-significant ( $P > 0.05$ ), implying homogeneous slopes between treatments (Wilkinson 1990).

*T. maculatus* parasitism on *M. edulis* is not limited to a decrease in mussel length. For a given size, bivalves harboring Stage IV-V female crabs showed significantly lower biomass than uninfested ones. This phenomenon was first reported in Argentina by

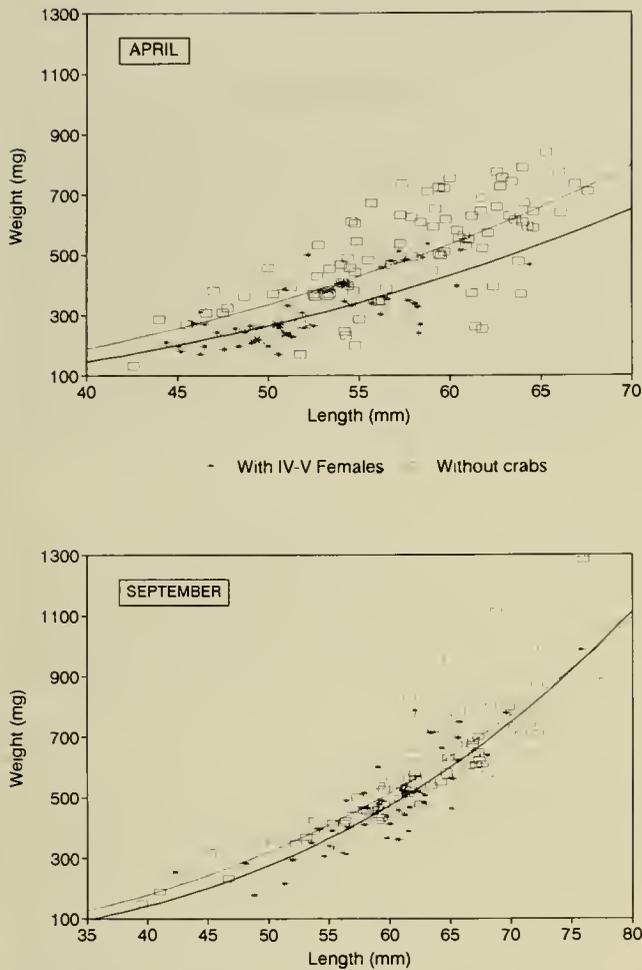


Figure 5. Length-weight relationship in *M. edulis* harboring Stage IV-V female *T. maculatus* and without pinnotherid crabs, April and September, 1990. Regression equations were back-transformed. Heavy line: with females (April: Weight = 0.0078 Length<sup>2.667</sup>, R<sup>2</sup> = 0.539; September: Weight = 0.0024 Length<sup>2.973</sup>, R<sup>2</sup> = 0.759). Thin line: without crabs (April: Weight = 0.0144 Length<sup>2.570</sup>, R<sup>2</sup> = 0.466; September: Weight = 0.0114 Length<sup>2.618</sup>, R<sup>2</sup> = 0.825).

Castellanos (1962), who observed incidentally that parasitized mussels were remarkably thin.

DISCUSSION

The infestation rate in Quequén Bank is similar to that found by Bierbaum and Ferson (1986) in a *T. maculatus* population off Massachusetts (69%), although Pearce (1964) had reported a rate of 97.6% for a neighboring locality. Infestation rate of *T. maculatus* in the scallop *Chlamys lischkei* Dunker in the San José Gulf, Argentina, was very low (1–12%) (Gómez-Simes 1993), as well as in *A. irradians concentricus* from North Carolina (Kruczynski 1972). *P. pisum* infestation rates in *M. edulis* varied from 4.5 to 46.4% in British intertidal banks (Seed 1969). Eighty percent of the mussels studied by Pregonzer (1978) in Australia were infested by *Pinnotheres hickmani* (Guiler).

Long-term studies are necessary to assess changes in pea crab infestation rates in bivalves. For instance, incidence of *Pinnotheres ostreum* Say in *Crassostrea virginica* Gmelin banks in Delaware Bay was 62.5% in June 1956, but dropped to just 28.9% two months later (Christensen and McDermott 1958).

The clumped distribution seen in *T. maculatus* pre-hard stages during the invasive stage is also characteristic of *P. ostreum* parasitizing *C. virginica* (Christensen and McDermott 1958). Present results indicate, however, that females are rarely found to share a host. The same was observed by Seed (1969) and Haines et al. (1994) in *P. pisum* infesting *M. edulis* in England. Male pea crab random distribution in *M. edulis* may be due to the fact that they are able to move freely from host to host (Orton 1920, Christensen and McDermott 1958, Pearce 1964), and probably can be regarded as occasional occupants. Present results indicate that pre-hard stages are less frequently found in mussels already occupied by adult crabs. Likewise, males are less frequent than expected in mussels already harboring a female. The opposite trend has been observed in *P. pisum* infesting *M. edulis* in England (Haines et al. 1994).

Present results agree with those of Seed (1969), since we have found a significant correlation of mussel length with female carapace width but not with male pea crab size. Bierbaum and Ferson (1986) reported that size of mussels and crabs inhabiting them were uncorrelated, but they did not distinguish between male and female crabs in their analysis. The smaller, more active male pea crabs change hosts frequently; therefore, a correlation between crab and mussel size was not expected. Female crabs, however, remain within the mussels over long periods of time, molting and growing together with their hosts. Likewise, growth of *P. hickmani* was directly related with that of its host (Pregonzer 1978).

Pea crabs living in the mantle cavity of bivalves feed on plankton-rich mucus that has been produced by their hosts (Orton 1920, Stauber 1945). Therefore, the host must spend energy filtering plankton that will be consumed by the crab. Filtration and oxygen consumption rates are affected by the presence of these true parasites (Bierbaum and Shumway 1988). In addition, damages have been often reported in gills, mantle walls and gonads (Stauber 1945, Christensen and McDermott 1958, Haven 1958, Dix 1973, Jones 1977). *T. maculatus* causes a distortion in shell shape char-

TABLE 5.

Covariance analysis of *M. edulis* dry weight without pea crabs or harboring *T. maculatus*, using mussel length as the covariate.

Source	SS	DF	MS	F	P
April 1990					
With IV-V Females					
vs. Without Crabs	1.477	1	1.477	22.67	<0.001
Error	9.315	143	0.065		
With Other Stages					
vs. Without Crabs	0.016	1	0.016	0.24	0.623
Error	12.368	190	0.065		
September 1990					
With IV-V Females					
vs. Without Crabs	0.192	1	0.192	7.13	0.009
Error	2.997	111	0.027		
Without Other Stages					
vs. Without Crabs	0.040	1	0.040	1.20	0.275
Error	3.521	105	0.034		

Statistical analyses were carried out on ln-transformed data. Slopes between treatments were always homogeneous. (Interaction treatment × covariate, P > 0.05). SS: sum of squares, DF: degrees of freedom, MS: mean square.

acteristic of low growth rates in *M. edulis* in the Atlantic coast of North America (Bierbaum and Ferson 1986). These authors reported, however, that lower growth rates occurred only in mussels harboring adult female pea crabs cultured in nutrient-poor environments. Kruczynski (1972) also reported that *A. irradians concentricus* individuals infested by adult female *T. maculatus* were smaller than uninfested scallops. Moreover, he demonstrated experimentally that growth rates were significantly lower in infested than in uninfested small- and mid-size scallops.

Effects of pea crab occurrence on bivalve weight has also been observed in other species. Irrespective of size, *A. irradians concentricus* individuals harboring *T. maculatus* had significantly lower dry weights than uninfested individuals (Kruczynski 1972). Pregoner (1981) found a significant decrease in condition and meat production in *M. edulis* individuals parasitized by *P. hickmani*, and soft tissue weight in *Mytilus californianus* Conrad was

also decreased when parasitized by *Fabia subquadrata* (Anderson 1975).

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## EFFECTS OF TRANSPORTATION STRESS AND RECOVERY AND SAMPLE TREATMENT ON $\text{Ca}^{2+}$ AND GLUCOSE CONCENTRATIONS IN BODY FLUIDS OF *ANODONTA ANATINA* (LINNAEUS)

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**ABSTRACT** Removal of lake mussels from a river and 15–20 minutes storage in river water in a bucket caused  $\text{Ca}^{2+}$  concentrations in the haemolymph and extrapallial fluid to increase significantly. Further storage and transport of mussels in a small quantity of river water to the laboratory increased the haemolymph and extrapallial fluid calcium concentrations, which were equal to each other and normally about  $4.5\text{--}6\text{ mmol L}^{-1}$  in summer, to nearly double, about  $8\text{--}12\text{ mmol L}^{-1}$ . In mussels maintained in the laboratory at  $4^\circ\text{C}$  for 2 weeks or 2 months, the elevated calcium concentrations decreased but often remained at about  $6\text{--}8.5\text{ mmol L}^{-1}$ . If mussels were returned to the river, the calcium concentrations were normalized. In the mantle cavity fluid,  $\text{Ca}^{2+}$  concentration (which normally was around  $4\text{ mmol L}^{-1}$ ) rose more slowly, but could reach that of the haemolymph after a transportation stress. During and after the transport of mussels, calcium leakage to the surrounding water was apparent (post-transport leakage of  $0.07\text{ }\mu\text{mol g}^{-1}\text{ h}^{-1}$  was measured over one week).

Glucose concentrations in mussel fluids showed a clear stress reaction when mussels were transferred to the laboratory. Haemolymph and extrapallial fluid glucose concentrations, which normally were very low ( $50\text{--}140\text{ }\mu\text{mol L}^{-1}$ ) rose about 5–7-fold, and that of mantle cavity fluid rose about 5-fold. In the laboratory, the elevated glucose concentrations decreased but tended to remain above normal. In a mussel group which had experienced additional stress, haemolymph and extrapallial fluid glucose concentrations remained at the 3-fold elevated level,  $300\text{--}320\text{ }\mu\text{mol L}^{-1}$ , for at least 2 weeks.

Storage of mussel fluid samples in a freezer significantly decreased measurable  $\text{Ca}^{2+}$  concentrations due to precipitate formation. Measurable glucose concentrations tended to increase (but not significantly) during as short a time as a few hours storage, particularly when no perchloric acid was added.

**KEY WORDS:** Lake mussel, *Anodonta anatina*, calcium, glucose, body fluids, transportation stress

### INTRODUCTION

The haemolymph calcium concentration in bivalves has frequently been studied with regard to acid stress or emersion (Malley et al. 1988, Pynnönen 1990, 1994, Byrne and McMahon 1991, Byrne et al. 1991). It is a well-known phenomenon that in bivalves experiencing respiratory, metabolic or mixed acidoses  $\text{CaCO}_3$  reserves are dissolved to buffer the protons and calcium concentration in the haemolymph increases. Fewer empirical studies have been done on the effects of stress on calcium concentrations in the extrapallial fluid or in the mantle cavity fluid. As early as 1935, Dotterweich & Elssner found that, during anaerobiosis, calcium concentration in the extrapallial fluid of *Anodonta cygnea* increased. They concluded that the extra calcium originated from the shell. Later, Crenshaw (1972) analysed ions in the extrapallial fluid of 3 marine bivalves and showed that the calcium concentration changed with the opening and closing of the valves. Heming et al. (1988) studied the effects of emersion and immersion in acid water on the composition of mantle cavity fluid in *Margaritifera margaritifera*. They found that also in the mantle cavity a carbonate buffer system operated, involving reactions with  $\text{CaCO}_3$  reserves of the mussel and release of  $\text{CO}_2$ .

Very little is known about the 'natural fluctuation' of  $\text{Ca}^{2+}$  concentrations in the fluids of bivalves because, in many experiments, control values used for comparison have been obtained from bivalves transported to a laboratory. Transportation conditions, as well as different types of intervention (even handling according to Dietz 1979) can disturb ionic concentrations in bi-

valve fluids. Due to the fact that experiments will, to an increasing degree, be carried out in a laboratory, it is very important to know how the transportation of bivalves to a laboratory affects their physiology.

The most obvious growing season of the shell of *Anodonta anatina* (L.) in a river in southern Finland is in midsummer but it is not known how long the annual growing season actually lasts (Pekkarinen 1991). In 1994, a study was begun into the seasonal variations in the calcium concentrations of the body fluids of *A. anatina* and how transportation and storage of mussels under laboratory conditions may affect the composition of the fluids. Some of the results have been published in a congress abstract (Pekkarinen and Suoranta 1994), and the seasonal cycle of calcium concentrations in the body fluids of *A. anatina* will be described in another paper (Pekkarinen 1995).

Because sampling of the extrapallial and mantle cavity fluids and subsequent treatment of all the bivalve fluids in the field immediately after emersion are somewhat problematic, different sampling methods were tested, and effects of aging and centrifugation on the fluids were investigated. It was hypothesised that the glucose concentration could be utilized to assess the purity of the fluids: it would be low in the extrapallial fluid and negligible in the mantle cavity fluid. It was discovered that the hypothesis was incorrect but there were indications that the glucose concentration might be useful in indicating stress in this species.

### MATERIALS AND METHODS

#### *Experimental Animals and Their Collection*

Lake mussels (*A. anatina*) were collected either by dragging the river bottom or, for 'natural' fluid analyses in the field, one at

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a time by hand from depths of about 0.3 to 0.5 m from the Vantaa River in Helsinki (southern Finland) in summer 1994. The sampling was done around noon. Calcium concentration in the river water varied between 13.5 and 19.5 mg L<sup>-1</sup> (0.34 and 0.49 mmol L<sup>-1</sup>) on different sampling days. The lengths and status of the mussels, dates of collection and sampling and river water temperatures are shown in Table 1. All parasitized or otherwise diseased (mainly with a pustular disease) (Pekkarinen 1993) mussels were excluded from the calcium and glucose calculations.

#### 'Natural' Bivalve Fluid Samples and Their Transport to the Laboratory

The mussel groups used in the 'normal analyses' are indicated with asterisks in Table 1. Sampling was begun within one minute of collecting a mussel. The mussel was wiped with tissue paper, and the haemolymph sample (0.4 to 0.9 mL) was taken with a 19 to 23 gauge needle, fitted to a 1 mL syringe, inserted between the valves into the sinus of the posterior adductor muscle.

Samples of the mantle cavity fluid were taken by forcing the shell valves apart slightly with a knife and allowing a small amount of fluid to drain into an Eppendorf tube.

At first, taking extrapallial fluid samples from unopened mussels by inserting a needle between the valves into the central extrapallial space of one valve was tried. This risked breaking the outer epithelium of the mantle, when the needle was moved in order to assess its position or to enhance free suction of fluid. It was also possible that the wound in the mantle could consequently tear and allow the mixing of mantle cavity fluid with extrapallial fluid. Later, samples of extrapallial fluid were taken after cutting the adductor muscles of the bivalve. Care was taken not to break the mantle of 1 valve. The mantle was wiped with tissue paper,

and paper was also used to prevent haemolymph from the adductor muscle entering the mantle cavity. The extrapallial fluid sample was taken by puncturing the mantle.

The fluid samples were transported to the laboratory in styrofoam boxes cooled with freezing blocks. The total time taken for sampling and transport was about 2 to 2.5 hours.

#### Treatment of the Fluid Samples

The fluid samples were first centrifuged (6000 rev · min<sup>-1</sup>, 5 minutes) in order to eliminate cells or mud or clay particles. Then, an equal volume of 0.33N perchloric acid was added to the sample aliquots for glucose analysis, and, after mixing, the samples were centrifuged as above. The supernatant was used for the analysis. The glucose analysis was done within 1 day of collection and the calcium analysis (from non-deproteinized supernatants) within a few days (the samples were kept in closed vessels at 4°C). Samples from a few mussel groups used for calcium analysis were stored frozen (-18°C), and a correction factor (Fig. 1) was used in the calculation. Both the colorimetric glucose and calcium analyses were done using kits (Calcium C code No. 997-21809, Wako Chemicals GmbH; Glucose GOD-Perid Methode, Boehringer, Mannheim GmbH).

#### Experiments on Sample Treatment

For sample treatment experiments, fluid analyses from mussel groups collected on June 20, July 22, July 25, July 28 and August 25 were used (Table 1). Detailed descriptions of the treatments are presented in Figures 1 and 2.

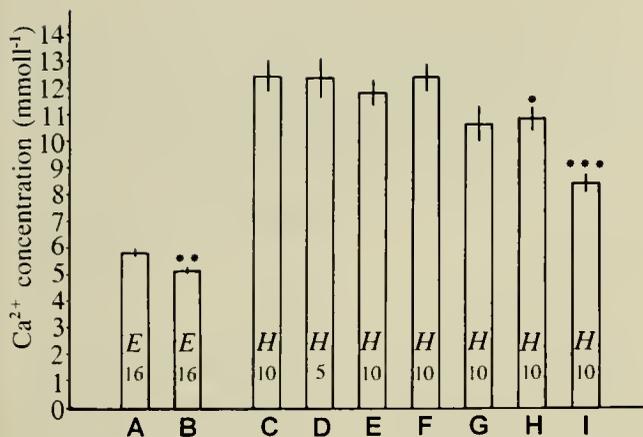
TABLE 1.

Lake mussels (*Anodonta anatina*) from the Vantaa River Used in the experiments of this study in summer 1994.

Group	Date of Collection	Sampling Date	Water t (°C)	All Mussels				Mussels Used in the Results			
				N	Length (mm)	Deleted Parasitized	Deleted Otherwise Diseased	Length (mm)	N	♂♂/♀♀	Gravid Mussels - or +
1*	1.7.	1.7.	17.5	7	78.5 ± 4.2	-	-	78.5 ± 4.2	7	7/0	-
2	20.6.	4.7.		8	83.8 ± 4.0	-	1	85.0 ± 4.5	7	7/0	-
3*	5.7.	5.7.	19.0	9	81.7 ± 2.0	-	1	82.2 ± 2.2	8	7/1	-
4	20.6.	5.7.		10	80.4 ± 3.8	-	1	80.1 ± 4.2	9	5/4	-
5*	22.7.	22.7.	22.0	24	81.5 ± 1.7	6 <sup>a</sup>	2	81.8 ± 2.2	16	13/3	+
6	22.7.	4.8.		10	77.1 ± 1.9	2	-	77.5 ± 2.4	8	4/4	+
7*	25.7.	25.7.	22.5	10	78.2 ± 3.1	-	1	78.3 ± 3.5	9	5/4	+
8	25.7.	2.8.		9	77.3 ± 3.4	-	3	78.9 ± 4.4	6	4/2	+
9*	28.7.	28.7.	23.0	10	78.4 ± 2.2	1	-	78.6 ± 2.5	9	5/4	+
10	28.7.	28.7.		10	77.2 ± 2.3	2	-	77.1 ± 2.5	8	2/6	+
11	28.7.	28.7.		11	75.0 ± 1.7	1	1	75.4 ± 1.8	9	5/4	+
12	28.7.	28.7.		9	76.2 ± 2.7	-	1	76.2 ± 3.1	8	6/2	+
13	28.7.	10.8.		10	76.9 ± 4.2	-	1	78.2 ± 4.5	9	7/2	+
14	20.6.	24.8.		10	65.8 ± 1.6	-	-	65.8 ± 1.6	10	8/2	-
15*	25.8.	25.8.	16.0	12	83.7 ± 3.2	-	1	84.6 ± 3.3	11	7/4	+
16	25.8.	25.8.		13	75.6 ± 2.5	3	1	75.2 ± 2.9	9	7/2	+
17	25.8.	9.9.		10	68.4 ± 2.6	-	1	69.7 ± 2.5	9	8/1	+
				182							

\* Mussel groups used for 'normal' analyses in the field.

<sup>a</sup> Parasitized mussels considered separately.



**Figure 1.** Effect of sample treatment on measurable  $\text{Ca}^{2+}$  concentration in the extrapallial fluid (E) and haemolymph (H) of *A. anatina* (results from groups 9–13 = first series and 6 = second series). (A) Refrigerated ( $4^{\circ}\text{C}$ ) extrapallial fluid centrifuged (6000rpm, 5 min) 2.5 hours after sampling and calcium measured from fresh supernatant. (B) Supernatant stored frozen ( $-18^{\circ}\text{C}$ ) and calcium measured after 7 weeks. (C) Haemolymph sampled in the laboratory and calcium measured immediately. (D) Calcium measured from the same haemolymph after 2.5 hours' storage in a refrigerator. (E) An aliquot of the haemolymph sample after 2.5 hours' storage centrifuged and calcium measured from the supernatant. (F) Calcium measured from the haemolymph after 1 week storage in the refrigerator. (G) The centrifuged supernatant has been stored in the refrigerator for 1 week before calcium analysis. (H) After 1 week's storage in the refrigerator (see F) haemolymph stored frozen for 6.5 weeks. (I) The supernatant (see G) stored frozen for 6.5 weeks. Numbers of samples are indicated in the lower parts of the columns. Comparison with A and C made with a 2-sided *t* test (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ ).

#### Transportation of Mussels to the Laboratory and Their Maintenance in the Laboratory

The mussels were placed in buckets containing small amounts of river water (totally or almost totally immersed), transported for about 1 km in a cart and then by bus to the laboratory. This was done during the summer and the temperature of the mussels could not be regulated during transportation. In the laboratory, the mussels were kept in a dark room (at  $4^{\circ}\text{C}$ ) in plastic 40-liter aquaria without sediment in running, charcoal-filtered, aerated tap water. The water calcium concentration was  $10.8\text{--}19.2\text{ mg L}^{-1}$  ( $0.270\text{--}0.479\text{ mmol L}^{-1}$ ). Several experiments were done during the summer and they are described as follows.

#### Recovery Experiment, June 20–July 5

The mussels in groups 2 and 4 (Table 1) had been kept in the laboratory from June 20 but the mussels in group 4 had been returned to the river on July 1. They were put in a net bag on the bottom of the river. The mussels from groups 2 and 4 were sampled on July 4th and 5th, respectively, and their calcium and glucose concentrations were compared with each other. 'Normal' calcium and glucose concentrations from groups 1 and 3 (on July 1 and July 5, respectively) (Table 1) were used for additional comparison.

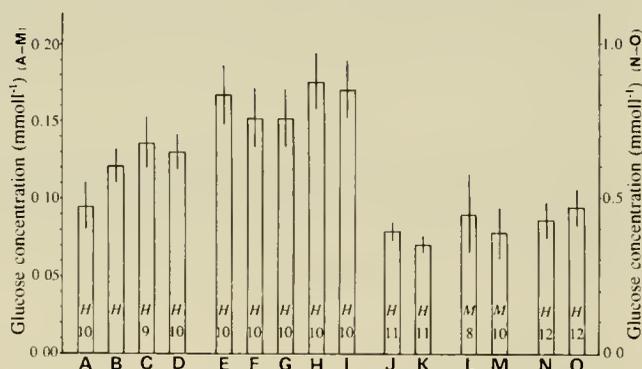
#### Experiment with Repeated Sampling and Effect of Sediment, July 22–August 4

Haemolymph samples (0.5 mL) from the individuals in group 5 were taken in the field on July 22 (first sampling,  $S_1$ ). These

mussels and another mussel group (6) were brought to the laboratory in a small amount of water (partially emersed). The mussels in group 6 were sampled in the laboratory after arrival. The mussels in group 5 were randomly divided into 2 subgroups,  $S_{5a}$  and  $S_{5b}$ , and were put into buckets,  $S_{5a}$  without sediment and  $S_{5b}$  with clay mud from the river bottom. Aerated, charcoal filtered water ( $4^{\circ}\text{C}$ ) was intermittently run over the mussels. Dead animals were removed when noticed. The mussels from the subgroups of 5 were sampled again on August 4 (second analysis,  $S_2$ ; all fluid samples).

#### Leakage Experiment, July 25–August 2

The mussels in group 8 were brought to the laboratory on July 25 and put in individual jars with charcoal-filtered tap water ( $5 \times$  mussel weight). The jars were not closed tightly and the water was not aerated. The calcium concentration in the water was measured 6 times during the 8-day experiment. The average amount of calcium leakage over 7 days was calculated by using the results from 6 healthy mussels. No more than two of the nine mussels survived for 8 days, and thus haemolymph samples could be taken only from these two. Initial events in mussel haemolymph calcium



**Figure 2.** Effect of sample treatment on measurable glucose concentration in the haemolymph (H) and mantle cavity fluid (M) of *A. anatina* (results from mussel groups 7, 14, 15 and 16, see Table 1; different experiment series separated by broader intervals). (A) Protein precipitant (0.33N perchloric acid) added immediately to haemolymph (1:1) in the field, and the sample centrifuged (6000rpm, 5 min) after transporting to the laboratory (2.5 hours) and glucose measured from the supernatant (stored at  $4^{\circ}\text{C}$ ) on the same day. (B) Haemolymph centrifuged after cold storage ( $4^{\circ}\text{C}$ ) for 2.5 hours, and perchloric acid added and supernatant used for glucose analysis. (C) The haemolymph supernatant (see B) stored 2 days in the refrigerator before addition of perchloric acid and glucose analysis from the supernatant. (D) The haemolymph supernatant (see B) stored frozen ( $-18^{\circ}\text{C}$ ) for 2 weeks before adding perchloric acid and glucose analysis. (E) Haemolymph from laboratory-maintained mussels diluted immediately with distilled water (1:1), and glucose measured. (F) Fresh 0.33N solution of perchloric acid added (1:1) to haemolymph and glucose measured from supernatant. (G) As F but perchloric acid solution several months old. (H) Haemolymph centrifuged after 2.5 hours' cold storage and diluted with distilled water (1:1) before glucose analysis. (I) As H but perchloric acid and centrifugation instead of addition of distilled water. (J) As H. (K) As I. (L and M) As J and K but mantle cavity fluid as material. (N) As B but mussels brought to the laboratory (y scale at the right). (O) Haemolymph supernatant stored in a refrigerator for 1 day before addition of perchloric acid and glucose analysis. Two-sided *t* test did not reveal significant differences in any test groups.

concentration were hypothesised from results of groups 7 and 12 (see Table 1 and next paragraph).

#### Handling Stress Experiment, July 28–August 10

Fluid samples from the mussels in group 9 were taken in the field as the 'normal' samples (immediately after collecting) (Table 1). The mussels of group 10 were allowed to rest in a bucket of river water for 15 to 20 minutes and the mussels from group 11 were allowed to rest for 1.5 hr before sampling. The mussels in groups 12 and 13 were then brought to the laboratory. The mussels from group 12 were sampled about 5 hr after collection (warmed to the ambient air temperature, about 27°C). The remaining mussels (group 13) were kept in an aquarium for 13 days and then sampled (10th of August).

#### Long-Term Laboratory Maintenance, June 20–August 24

The mussels (group 14) were kept in the laboratory from June 20 to August 24.

#### Late Summer Experiment; Experiment Particularly to Study the Effect on Glucose Concentration, August 25–September 9

The mussels in group 15 were sampled in the field (August 25), those in group 16 at arrival at the laboratory and the mussels in group 17 after keeping in the laboratory for 15 days (on September 9).

#### Statistical Analyses

The results of measurements are presented as mean  $\pm$  SEM. The calcium and glucose concentrations in different groups were compared with a 2-sided *t* test. *p* values  $< 0.01$  were considered as significant.

## RESULTS

#### Sample Treatment

When samples of extrapallial fluid (kept at 4°C) were centrifuged 2.5 hours after collection and some aliquots of the supernatants were immediately used for calcium determination and other aliquots were stored at  $-18^{\circ}\text{C}$  for seven weeks before calcium determination, it was found that the measurable calcium concentration decreased significantly ( $p < 0.01$ ) during freeze-storage (Fig. 1A,B).

Apparent calcium concentrations in non-centrifuged haemolymphs did not change during either the 2.5 hour or 1 week storage of the fluid at 4°C (Fig. 1C,D,F). An additional storage period (6.5 weeks) at  $-18^{\circ}\text{C}$  somewhat decreased the calcium concentration ( $p < 0.05$ ) (Fig. 1H).

If the haemolymph was centrifuged after a 2.5 hour storage time (to simulate the waiting time normally resulting from the sampling of a group of mussels and the transportation of the samples to the laboratory) and calcium was measured in the cell-free supernatant, the concentration showed a slight decrease (Fig. 1E) but the decrease was not significant even after 1 week's storage of the supernatant at 4°C (Fig. 1G). If the supernatant was stored at 4°C for 1 week, kept at  $-18^{\circ}\text{C}$  for 6.5 weeks and then the calcium was measured, a very significant decrease ( $p < 0.001$ ) was observed (Fig. 1I). Frozen samples had become turbid and a precipitate was observed on the inner surfaces of the vessel walls.

Apparent glucose concentrations in the fluids tended to in-

crease, although not significantly, during the storage of the fluids at 4°C as native samples or the storage of deproteinized supernatants from the fluids (Fig. 2). The highest increase was apparent within the first few hours after sampling. Immediate addition of the deproteinizing agent (0.33 N perchloric acid, 1:1) to haemolymph prevented the rise in glucose concentration.

#### Effects of Transporting Mussels to the Laboratory and Maintenance in the Laboratory

##### Calcium Concentrations

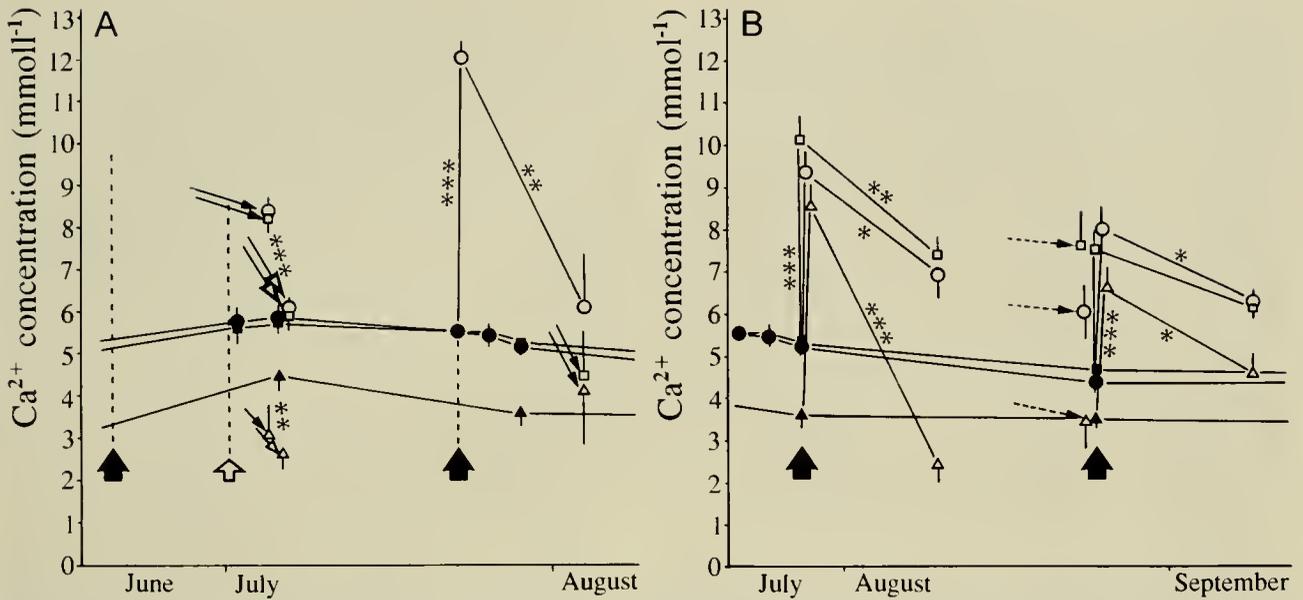
Throughout the study, calcium concentrations in the haemolymph and extrapallial fluid did not differ from each other. Instead, in most mussel groups there was a significant positive correlation between them.

Transportation of the mussels to the laboratory resulted in about 2-fold higher calcium concentrations in the haemolymph and extrapallial fluid (see the  $\text{Ca}^{2+}$  increases at the sites pointed to by bold black arrows in Figs. 3A,B). Keeping the mussels in the laboratory for 2 weeks only partially normalized these increased values (Figs. 3A,B). The calcium concentration in the mantle cavity fluid was usually significantly lower than that of the haemolymph but clearly higher than that of the surrounding water. During the transportation, the calcium concentration in the mantle cavity fluid rose to similar levels as in the haemolymph (Fig. 3B), and, during the maintenance of the mussels in the laboratory, it decreased to near normal level, with some indications of overshooting in the experiments in early July (Fig. 3A) and early August (Fig. 3B). Even after 2 months' maintenance of mussels in the laboratory, the calcium concentrations in the haemolymph and extrapallial fluid remained above the normal level (Fig. 3B, broken arrows).

When the mussels were returned to the river in a net bag, the increased calcium concentrations in the haemolymph and extrapallial fluid returned to normal but decreased significantly below the normal value ( $P < 0.01$ ) in the mantle cavity fluid (Fig. 3A, arrows with white-cored heads).

In the mussel group for repeated sampling (group 5), there were originally in the first ('normal') sampling (the second bold black arrow in Fig. 3A) 24 animals. Later, dissection revealed that there were 6 parasitized mussels and two suffered from a severe pustular disease (Table 1). Although the mean haemolymph calcium concentration among 5 parasitized mussels did not differ from that of 16 healthy mussels, all parasitized mussels were excluded from the results throughout the whole study.

Thirteen mussels in group 5 survived to the second sampling after about 2 weeks (Fig. 3A, early August); the immediate after-transport maximal value was obtained from an additional group (group 6). Of the parasitized mussels, two survived and both diseased mussels survived. There were only 9 healthy mussels remaining to be used in the comparison of the first and second samples. Group 5<sub>2</sub> was further divided into 2 subgroups (5<sub>a</sub> kept without sediment and 5<sub>b</sub> kept in natural river sediment in the laboratory), and the numbers of healthy mussels surviving in these subgroups were five and four, respectively. Calcium concentrations in the haemolymph and the extrapallial fluid were slightly higher (but not significantly) in the group without sediment. It has to be noted that in all of group 5<sub>2</sub> calcium concentrations were very variable: between 1.66 and 15.38 mmol L<sup>-1</sup> in the haemolymph

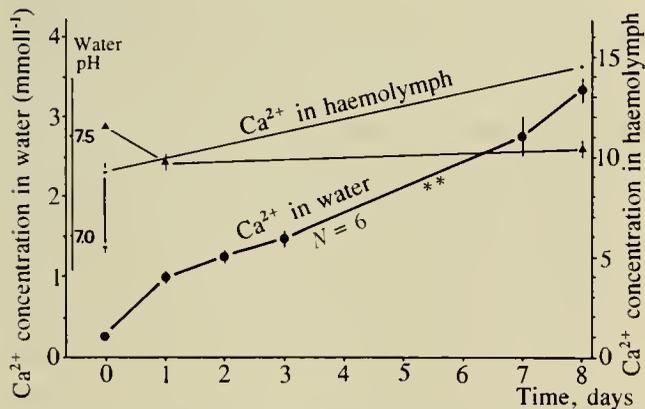


**Figure 3.** Effects of transport to (bold solid arrows) and maintenance of *A. anatina* in the laboratory on calcium concentrations in its different fluids in midsummer (A) and late summer (B). Solid symbols (● = haemolymph, ■ = extrapallial fluid, ▲ = fluid in mantle cavity) indicate values measured in the field and open symbols, respectively, values measured in the laboratory (means with one SEM or two, or sometimes SEMs are hidden by the fluid symbols). Hypothetical courses are indicated with thin arrows (in long-term experiment with broken thin arrows, Fig. 3B, August). The values measured after returning mussels to the river (Fig. 3A, open bold arrow) are indicated with thin arrows with white-cored heads (in early July). Comparison of concentrations was made by 2-sided *t* test (\* = *p* < 0.05, \*\* = *p* < 0.01, \*\*\* = *p* < 0.001).

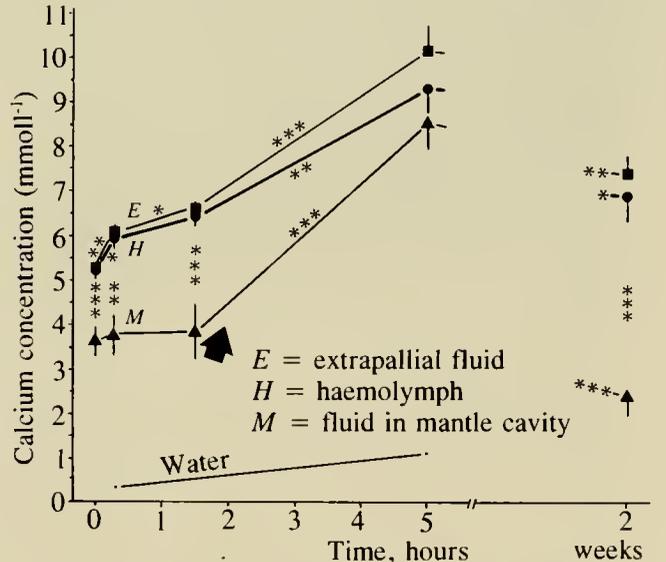
(reflected in the large SEMs in Fig. 3A, the last values of the graph). In the parasitized mussels, the variation was similar.

In a transportation experiment (group 5), it was noticed that mussels leaked calcium into the transporting water. Therefore, a separate leakage experiment was done (using mussel groups 7, 8 and 12) (Fig. 4). After transporting the mussels (group 8) to the laboratory, the calcium concentration in the water (5 × mussel weight) in 6 jars (1 mussel per jar) increased tenfold, from 0.27

mmol L<sup>-1</sup> to 2.77 ± 0.26 mmol L<sup>-1</sup> by day 7 and to 3.33 ± 0.16 mmol L<sup>-1</sup> by day 8. During 7 days, the mussels leaked calcium into the water at a rate of 485 ± 36 μg g<sup>-1</sup> h<sup>-1</sup> (0.072 μmol h<sup>-1</sup>). Only 2 mussels survived until day 8, and their haemolymph



**Figure 4.** Calcium leakage experiment (mussel group 8). Change of calcium concentration in 5-fold mussel weight of stagnant (charcoal-filtered, 4°C) tap water during 8 days post mussel transport to the laboratory. Initial events in mussel haemolymph calcium concentration were hypothesized from results of groups 7 and 12. Mussel mortality in group 8 was greatest around day 7; the haemolymph calcium value in day 8 is from 2 surviving mussels.



**Figure 5.** Handling stress experiment. Change of calcium concentrations in fluids of *A. anatina* as a function of time after collecting the mussels from the river and keeping them in river water in a bucket (groups 9–11) and after transporting (bold solid arrow) them (group 12) to and maintaining them in the laboratory (at 4°C) for 2 weeks (group 13). Calcium concentration was also measured in the transporting water (the lowest line). Statistical analyses as in Figure 3.

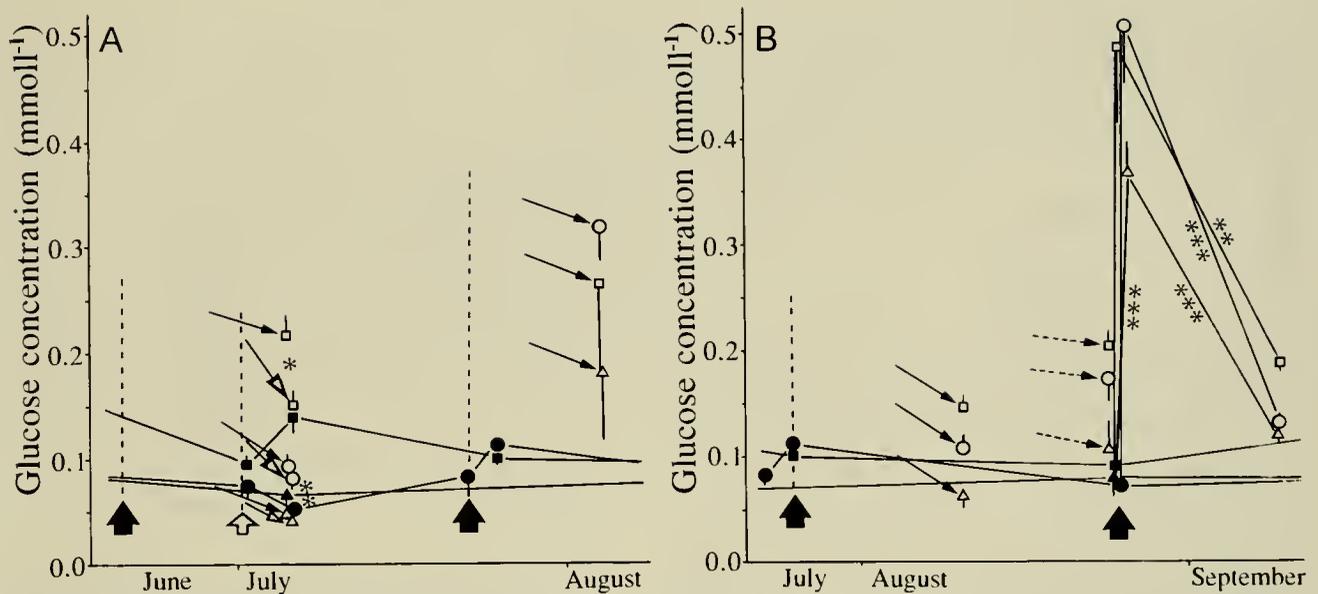


Figure 6. Effects of transport to (bold solid arrows) and maintenance in the laboratory of *A. anatina* on glucose concentrations in its different fluids (● = haemolymph, ■ = extrapallial fluid, ▲ = fluid in mantle cavity) in midsummer (A) and late summer (B). Hypothetical courses are indicated with thin arrows. Bold open arrow = returning of mussels to the river; the values after it are marked with thin arrows with white-cored heads. See Figure 3.

calcium concentrations were 11.63 and 16.82 mmol L<sup>-1</sup>. The mussels died with the foot extended.

The handling stress experiment (using mussel groups 9–13) (Fig. 5) was done in order to see whether just collection and 15–20 minute waiting time in a bucket of river water affected the calcium concentrations in the mussel fluids and how fast the calcium concentrations rose. A waiting time of 15–20 minutes caused a significant increase in the calcium concentrations of the haemolymph and extrapallial fluid, and further waiting and transporting of the mussels caused further increase in the concentrations. The calcium concentration in the mantle cavity fluid did not increase until transportation, and, after the transportation, it was as high as that of the haemolymph. The calcium concentration in the transporting water rose from 0.34 (concentration in the river water) to 1.14 mmol L<sup>-1</sup>. After a 2-week maintenance of mussels in the laboratory, the calcium concentration in their mantle cavity fluid had decreased to below the concentration measured at the beginning of the experiment ( $p < 0.05$ ) (Fig. 5). The same experiment is summarized in Figure 3B (late July–early August).

#### Glucose Concentrations

In an experiment in late summer 1994 (Fig. 6B, late August–early September), glucose concentrations in the haemolymph and extrapallial fluid rose to 5–7-fold during transport of mussels to the laboratory. In the fluid of the mantle cavity, a very significant increase in glucose concentration was also observed. After keeping mussels in the laboratory for 2 weeks, glucose concentrations in the haemolymph sometimes remained above normal values (Fig. 6A, both experiments; and 6B, second experiment) and sometimes approached the original values (Fig. 6B, first experiment). In the experiment with repeated sampling (Fig. 6A, late July–early August), the glucose concentration remained 3 times the original value. Even after a 2-month maintenance of mussels in the laboratory, haemolymph glucose concentration was significantly higher than that of mussels in the wild (Fig. 6B, broken

arrow in late August). In the extrapallial fluid of mussels, particularly, glucose concentration remained at an elevated level after 2-week or 2-month maintenance of the mussels in the laboratory (Figs. 6A and 6B, all experiments). Returning of mussels to the river enhanced restoration of the normal glucose concentrations in the haemolymph and extrapallial fluid (Fig. 6A, arrows with white-cored heads).

## DISCUSSION

### Sampling of the Fluids and Their Treatment

Haemolymph samples of bivalves are usually taken from either the pericardium or the sinuses of the adductor muscles or the foot (Crenshaw 1972, Fyhn and Costlow 1975). In this experiment, the haemolymph samples were taken from the sinus of the posterior adductor muscle. It is possible that, at the puncture site, some haemolymph can leak and run over the adductor muscle into the mantle cavity and contaminate the mantle cavity fluid. Also the forceful pulling apart of the shells in order to drain the mantle cavity fluid can break mantle tissue resulting in contamination by some haemolymph and can expose the marginal extrapallial space. The fluid volume in this space is, however, minimal. Mucus from the mantle surfaces may accompany the mantle cavity fluid. Horohov et al. (1992) also considered drainage of the mantle cavity fluid of *Dreissena polymorpha*, although providing a large volume, problematic. They observed that failure to drain the mantle cavity fluid in smaller zebra mussels could contaminate the haemolymph sample if the syringe needle passes beyond the pericardial region. The extrapallial fluid of *A. anatina* was collected last, through the mantle. Moreover, the attachment sites of the mantle to the shell have to be intact, or otherwise the extrapallial fluid may be contaminated by haemolymph or mantle cavity fluid.

As early as 1930, de Waele found that exposure of the blood of *Anodonta cygnea* to air caused precipitation of a thin film of calcium carbonate and protein. This is not surprising since bivalve

haemolymph is saturated with respect to aragonite (Potts 1954). De Waele (1930) stated that the precipitate formed as a result of loss of carbon dioxide leading to a rise in pH. The fluids of *A. anatina* tended to become somewhat cloudy during storage, particularly when stored frozen. Crenshaw (1972) found a similar phenomenon in fluids of marine bivalves even 10 minutes after the fluid collection. Attempts by Crenshaw to clarify the samples by centrifugation or by pressure or vacuum filtration were only partially successful. Calcium concentrations in these partially clarified samples were lower than in the samples diluted immediately after their collection. Crenshaw also studied non-dialysable macromolecules (protein and mucopolysaccharides) of the mussel fluids, and he suggested that, in the extrapallial fluid, calcium is bound to glycoprotein. In this present study, the mussel fluids were centrifuged (to remove possible contamination by cells) about 2.5 hours after sampling, and, therefore, some calcium may have been lost if it had precipitated during this time. The calcium concentration of haemolymph did not, however, decrease significantly during this period. In a later experiment in winter (unpublished), it was found that the calcium concentration in extrapallial fluid did not change if it was measured either immediately after sampling and centrifugation or after a 2.5 hour delay and centrifugation. Centrifugation was particularly important for the mantle cavity fluid samples, because there was often mud in the cavity. Because storage of frozen supernatants of mussel fluids caused a significant loss of calcium due to precipitation on the inner wall of the storage vessel, calcium analysis after freeze-storage of mussel fluids cannot be recommended.

Stored mussel fluid samples showed slightly but not significantly increased glucose concentrations. This is in accordance with the observations by Wijsman and Maaskant (1982) on glucose contents in stored tissues of *Lymnaea stagnalis*. Glycoproteins, mucopolysaccharides, or even glycogen contamination from the punctured adductor muscle or the mantle, may be responsible for this. Therefore, a protein precipitant should be added to the sample as soon as possible. Perchloric acid, in addition to removing glycoproteins, denatures enzymes which could release glucose.

#### *Transportation of Mussels and Their Maintenance in the Laboratory: Effect on Calcium Concentrations*

If freshwater mussels experience metabolic, respiratory or mixed acidosis, for instance as a consequence of emersion,  $\text{CaCO}_3$  reserves are then dissolved to buffer the protons (Dotterweich and Elssner 1935, Byrne and McMahon 1991, Byrne et al. 1991). Both  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$  increase in the haemolymph. The ratio  $\Delta\text{HCO}_3^-/\Delta\text{Ca}^{2+}$  depends on whether mussels are ventilating or not.  $\text{CO}_2$  formed from  $\text{HCO}_3^-$  passes freely out from an open mussel but extra  $\text{Ca}^{2+}$  remains for a longer time. It has been thought that the  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$  are taken from the shell (Dotterweich and Elssner 1935, Crenshaw and Neff 1969, Byrne and McMahon 1991, Byrne et al. 1991,) or from the calcified concretions which freshwater bivalves generally have in their tissues (Machado et al. 1988). The concretions are, however, mostly composed of calcium phosphate (in addition to an organic matrix) (Silverman et al. 1983, Pynnönen et al. 1987, Pekkarinen and Valovirta 1994), and only a small portion of mantle calcium is exchangeable (Jodrey 1954, Istin and Maetz 1964).

During transportation, the mussels may have been partially emersed due to the small amount of water, and the shells may have

been partially closed in response to the vibratory stress. The water temperature also rose to near that of the surrounding air. The calcium concentrations in the haemolymph and extrapallial fluid increased concomitantly. This is in accordance with the finding of Coimbra et al. (1993) that *A. cygnea* equilibrates calcium concentration between the haemolymph and the extrapallial fluid. Calcium concentration in the mantle cavity fluid of *A. anatina* is significantly lower than that in the haemolymph, but clearly higher than that of the surrounding water. This suggests that the mantle cavity is a compartment partially separated from the surrounding water. Matsushima and Kado (1982) also found that mantle cavity fluid of *Anodonta woodiana* was more similar to blood than pond-water. Because Scheide and Dietz (1984) reported that they had observed blood even being expelled from the extended foot of *Ligumia subrostrata*, especially when the mussels were handled, it is possible that the mantle cavity fluid of *A. anatina* contained haemolymph. During transportation, the mussels leaked additional calcium into the mantle cavity and so the calcium concentration in the mantle cavity fluid became close to that in the haemolymph. Mussels are thought to actively take in calcium through some epithelia (Coimbra et al. 1993). Specialized cells in the gill have been attributed to ion transport (Dietz and Findley 1980, Kays et al. 1990). Calcium moves to the extrapallial space by diffusion, and the epithelial cells of the shell side of the mantle are an order of magnitude more permeable to  $\text{Ca}^{2+}$  than the cells bordering the mantle cavity (Kirschner and Sorenson 1964, Coimbra et al. 1988). This would have the effect of conserving calcium. The permeability barrier of the outer mantle epithelium (Neff 1972) may be modulated seasonally (Coimbra et al. 1988). In the handling experiment, a small lag time in the rise of the calcium concentration in the mantle cavity fluid may have resulted from a permeability barrier.

The calcium concentrations in the haemolymph and extrapallial space of the mussels resting in a bucket of river water rose in only 15 minutes. Whether the extra calcium originated from the shell or from the calcified granules in the tissues is not known. Dietz (1979) also found that in *Margaritifera hembeli* acclimated to pond water in the laboratory and transferred to a small container of pond water (100 mL), sodium influx increased over several hours and then stabilised to normal. Thus minimal handling stress is enough to change ionic concentrations in fluids of some bivalves. Serotonin and cAMP are involved with regulation of sodium (Dietz et al. 1982, 1985), and sodium transport shows both seasonal and diurnal changes (Nemcsók and Szász 1975, Graves and Dietz 1980). Endogenous and exogenous calcium concentrations can also interfere via effects on adenylate cyclase (Scheide and Dietz 1984). Diurnal changes were excluded during this study by sampling mussels at the same time of the day. It has also been shown that the pH and calcium concentration in the extrapallial fluid of clams and mussels fluctuates with the opening and closing of the valves (Crenshaw and Neff 1969, Crenshaw 1972, Gordon and Cariker 1978). In the extrapallial fluid of *Mercenaria mercenaria*, changes in calcium concentration and pH were observable, reflecting a 10 to 15 minute opening-closing rhythm (Crenshaw 1972). This rhythm could not be taken into consideration in the present study. In the *A. anatina* in this study, the mean calcium concentration in the mantle cavity fluid did not change during the mussels' 1.5-hour wait in a bucket of river water (handling experiment). This means that there was not yet any net leakage of calcium from the mussels, or that the mussels excreted any accumulated calcium from the mantle cavity. The concomitant increase

of calcium concentration both in mantle cavity fluid and surrounding water during the transportation of the mussels suggests net leakage and only partial shell closure.

Leakage of calcium from post-transport *A. anatina* in a leakage experiment was of a similar order as for *M. hembeli* recorded by Heming et al. (1988) after exposure of the mussels to acid water. During 1 week, average leakage from *A. anatina* was  $0.07 \mu\text{mol h}^{-1} \text{g}^{-1}$  (total fresh weight) and from *M. hembeli*  $0.03 \mu\text{mol h}^{-1} \text{g}^{-1}$  (the leakage from *M. hembeli* was recorded in acid water, pH 5.25). In *A. anatina* the most rapid leakage occurred during the first day after transporting the mussels to the laboratory. The net efflux of calcium may have partially resulted from hampering the mussel's active calcium uptake by the low water temperature in the laboratory. *A. grandis simpsoniana*, after a 6-day emersion, showed a substantial calcium efflux during reimmersion,  $5.85 \mu\text{mol}^{-1} \text{h}^{-1}$  per g soft part dry weight (Byrne and McMahon 1991) ( $0.6 \mu\text{mol g}^{-1} \text{h}^{-1}$ , assuming that soft part dry weight is 20% of fresh weight and fresh weight is 50% of total mussel weight) (Pekkarinen 1993).

Machado et al. (1988) found that keeping *A. cygnea* in acidic water led to the formation of a calcified pellicle on the inner surface of the shell. This indicates that, in freshwater unionaceans, calcium might be taken from the calcified concretions of the tissues for buffering. On the other hand, it has been assumed that protons are pumped into the extrapallial fluid during acidosis (Machado et al. 1990, Hudson 1993) leading to dissolution of the shell. The exact time when the calcified pellicle is formed is not known. After stress, for instance after emersion or acid exposure, bivalves may experience a transient alkalosis (Byrne and McMahon 1991, Pynnönen 1994).  $\text{CO}_2$  is easily ventilated even from partially exposed mantle edges but the elevated calcium concentration persists longer, leading to imbalance of ions. Therefore extra calcium has to be eliminated from the haemolymph. Part of it may leak into the surrounding water and part may be precipitated onto the shell. Increased ventilation movements may have caused slight over-shooting of the decrease in calcium concentration in the mantle cavity fluid in some of the experiments in this study. This over-shooting was more pronounced in the mussels which were returned to the river.

In mussels kept in the laboratory for 2 weeks, calcium concentrations only partially returned to normal. The degree of normalization may depend on the season, the stage of reproductive cycle and the extent of stress experienced by mussels. In an experiment which was done when the river water temperature was highest and female mussels were at the beginning of gravidity, the mussels were punctured twice, and this may also have, due to leakage of haemolymph from the wound, increased mortality. Calcium concentrations in the survivors varied greatly: high in some and in others, possibly moribund individuals, very low.

The mussels in most experiments in this study were kept in

aquaria with no sediment in which to burrow. This may have stressed the mussels along with the very low, unseasonal temperature. Heat and cold stresses have been shown to change serotonin concentrations in the mussel central nervous system (Stefano et al. 1978). However, Pekkarinen has noticed that clams (*Macoma balthica*, unpublished) can be maintained in the laboratory for longer periods more successfully at lower temperatures.

#### *Transportation of Mussels and Their Maintenance in the Laboratory: Effect on Glucose Concentrations*

There is evidence that molluscs have hyperglycemic neurohormones (Lubet et al. 1976, Hemminga et al. 1985, Robbins et al. 1990). In an arcid clam, during anaerobiosis, the blood plasma glucose concentration rose from  $86 \mu\text{mol L}^{-1}$  to  $228 \mu\text{mol L}^{-1}$ , or even to  $500 \mu\text{mol L}^{-1}$ , during 96 hr of anaerobiosis (de Vooy et al. 1991, de Zwaan et al. 1995). The blood glucose of sea hares (*Aplysia dactylomeda*) responded to relatively small temperature or salinity changes or to short duration air exposures (Carefoot 1994). Even handling for blood sampling caused hyperglycemia 30–40 minutes after sampling in a snail: the blood glucose rose about 6.5-fold within 40 minutes (Marques and Falkmer 1976). A similar rise was found in the haemolymph and extrapallial fluid glucose concentrations of *A. anatina* during transportation in this study. The rise may partially result from handling stress, hypoxia and temperature change.

It has to be noted that glucose concentrations in the haemolymph and extrapallial fluid may remain above the normal level in mussels maintained in the laboratory. In this study, the temperature in the laboratory was low and the mussels were maintained without sediment and food in darkness. Blood sodium in bivalves is regulated by serotonin through cAMP (Scheide and Dietz 1984). Sodium is involved in glucose transport at least in the gills of *Crassostrea gigas* (Bamford and Gingles 1974). Such factors (e.g., temperature) which affect the sodium pump may also affect glucose metabolism. Constant light has been shown to dampen the diurnal variation of sodium net flux in *Corbicula fluminea* and it may also dampen the entire metabolic level (McCorkle-Shirley 1982). In this study the mussels were kept in constant darkness, and the effects of prolonged darkness are not known. Starvation and anaerobiosis are known to decrease exogenous glucose utilization while glycogen degradation increases (Zaba et al. 1981).

The glucose values obtained for *A. anatina* in the field analyses in this study were very low compared with the posttransport values of the same species or with blood sugar concentrations reported in many other molluscs (Goddard and Martin 1966). Thus any stressed state of laboratory-kept mussels has to be taken into account if physiological experiments are to be done with such mussels. *A. anatina* could be a good bivalve model to study stress effects and the regulation of glucose concentration.

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## INDUCTION OF SETTLEMENT AND METAMORPHOSIS IN THE TROPICAL OYSTER, *CRASSOSTEA BELCHERI* (SOWERBY), BY NEUROACTIVE COMPOUNDS

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**ABSTRACT** Larvae of the tropical oyster *Crassostrea belcheri* set and metamorphosed when exposed to epinephrine (EPI), norepinephrine (NE), L-3,4-dihydroxyphenylalanine (L-DOPA) and gamma-amino butyric acid (GABA). The optimum concentration of these neuroactive compounds was  $10^{-5}$  M when larval set increased from 20% in the controls to between 60–80%. Larval setting performances were generally depressed when exposure was extended to 24 hours compared to 1 hour. These results extend our knowledge of induced settlement in tropical oyster species and provides a useful technique for enhancing seed production in tropical bivalve hatchery operations.

**KEY WORDS:** Induction, settlement, metamorphosis, oyster, neuroactive compounds, *Crassostrea belcheri*

### INTRODUCTION

Swimming larvae of many benthic marine bivalves remain in the plankton for a period of time before undergoing metamorphosis into the juvenile stage. This metamorphic event usually occurs in conjunction with settlement.

The settlement response in numerous bivalve larvae is regulated by intrinsic and extrinsic factors, including heredity, age, nutritional history of the larvae as well as physical and chemical characteristics of available substrate (Hatfield 1984).

Previous studies on oyster larvae have shown that neuroactive compounds like epinephrine, norepinephrine, L-3,4-dihydroxyphenylalanine and gamma-aminobutyric acid have influenced setting and metamorphosis in *Crassostrea gigas* (Coon et al. 1985, 1986, Cooper 1983, Cooper and Shaw 1984), *C. virginica* (Walch et al. 1988) and *Ostrea edulis* (Shpigel et al. 1989). However, nothing has been reported on the effects of neuroactive compounds on the setting of the tropical oyster *Crassostrea belcheri*. The aim of this study was to test the setting response of *C. belcheri* larvae towards neuroactive compounds, as an attempt to optimize the setting performance of tropical oyster larvae in the hatchery.

### MATERIALS AND METHODS

Larvae of *C. belcheri* were obtained using standard culture techniques described in Wong et al. (1989). Eyed larvae of *C. belcheri* were cultured using 1  $\mu$ m filtered seawater (18 ppt). Culture media were changed on alternate days. A prescribed diet of *Isochrysis galbana* (Tahitian strain) was provided daily. Metamorphic competence was ascertained by the presence of well-developed eyes and a predilection to metamorphose on the surfaces of the holding tanks or on cultch provided.

Competent eyed larvae collected in a 253  $\mu$ m nitex sieve were used in all experiments. The neuroactive compounds tested were epinephrine (EPI), norepinephrine (NE), L-3,4-dihydroxyphenylalanine (L-DOPA) and gamma-amino butyric acid (GABA) at concentrations of  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  M.

The experiments were conducted in plastic tissue culture plates

(24 wells; Falcon #3047) using 1  $\mu$ m filtered seawater mixed with fresh water (salinity: 18 ppt) at room temperature (26–28°C). EPI and NE were dissolved in 0.0005 N hydrochloric acid while L-DOPA and GABA were prepared as  $10 \times$  stock solutions in distilled water. For experiments, the stock solutions were diluted (1:9) in seawater to achieve the desired final experimental concentrations.

All test treatments were conducted in triplicate. In each experimental replicate, 50–100 larvae were placed in a total volume of 2 mL of test solution. A few washed marble chips measuring 3–4 mm in diameter were placed at the bottom as cultch. Larvae were exposed to various concentrations of the test compounds for 1 hour or 24 hours, then removed, rinsed and placed in fresh 18 ppt medium for the remainder of the observation period (24–48 hours). Food was not provided during the experiment. Controls were kept in 18 ppt with the same number of marble chips. In all experiments, the larvae were examined regularly with a dissecting microscope to monitor behaviour patterns or transient-responses to the test compounds.

At the end of each experiment, all wells were examined to determine the percentage of the total number of larvae which had set and/or metamorphosed. A larva was categorized as metamorphosed if it showed significant new shell growth. Spat that could not be washed off by using a water jet from a Pasteur pipette were recorded as "cemented." The experimental procedures were modified from those reported in Coon et al. (1985).

All statistical comparisons were conducted using one-way analysis of variance at a 95% level of significance. If statistical significance was noted, then a Duncan's Multiple Range Test was conducted to determine which treatments were significantly different.

### RESULTS

Observations on the behavioural responses of *C. belcheri* eyed larvae when exposed to the various test compounds showed that they were similar to those reported by Coon et al. (1985) for *C.*

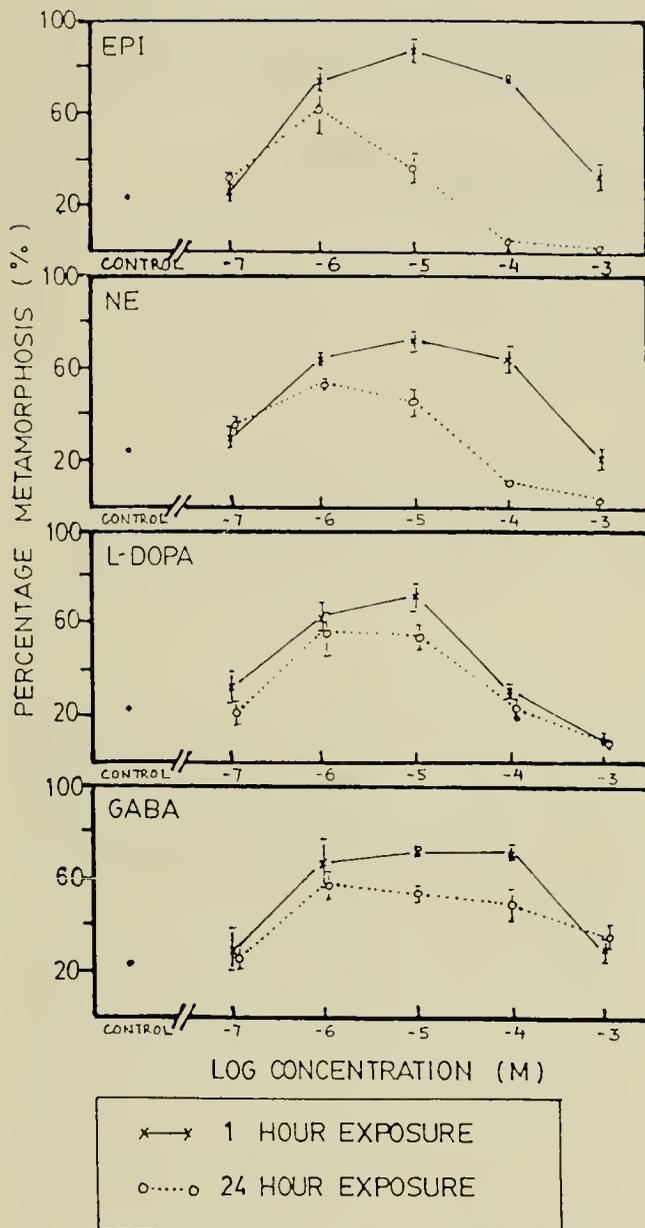


Figure 1. The percentage of *Crassostrea belcheri* eyed larvae which metamorphosed in response to 1 hour and 24 hour exposures to various concentrations of test compounds.

*gigas*. On exposure to EPI and NE, the larvae sank to the bottom of the well and immediately began morphogenetic differentiation. On the other hand, larvae exposed to both L-DOPA and GABA swam with their foot extended followed by crawling along the substratum. Subsequently, they cemented themselves and underwent metamorphosis.

Figure 1 shows the percentage of eyed larvae which had metamorphosed (when scored 4 days later) after 1 hour and 24 hours exposure to various concentrations of the test compounds. Setting performance was generally depressed when larvae were exposed for 24 hours, sometimes to levels lower than controls. In the case of 1 hour exposure, all 4 compounds increased the portion of larvae setting, with the best concentration at  $10^{-5}$  M.

Details of statistical analysis on the setting performances for 1 hour and 24 hours exposure are summarised in Tables 1 and 2 respectively. The highest larval setting occurred in EPI at  $10^{-5}$  M ( $87.0 \pm 5.3\%$ ). This was significantly higher ( $P > 0.05$ ) than those recorded for NE, L-DOPA and GABA at the same concentration ( $70.1$ – $71.8\%$ ). Concentrations of L-DOPA above  $10^{-4}$  M caused a variety of toxic effects including reduced larval activity, withdrawal of all tissues into the larval shell and increased mortality.

Figures 2 and 3 show the relative proportion of spat which were cemented after 1 hour and 24 hours exposure to various concentrations of the test compounds. EPI at  $10^{-4}$  M yielded the highest percentage of free (cultchless) spat followed by EPI at  $10^{-5}$  M and NE at  $10^{-4}$  M. In comparison, the yield of free spat was negligible with L-DOPA and GABA. Once again, exposure for 24 hours resulted in lower number of free spat.

## DISCUSSION

The result of this study demonstrates that larvae of the tropical oyster, *C. belcheri* can be induced to set by exposure to the compounds EPI, NE, L-DOPA and GABA. Exposure for 1-hour period increased larval setting success up to 3.5 times that controls, but prolonged exposure to some of these resulted in reduced effects sometimes to levels below controls.

The optimum concentration of EPI for larval settlement in *C. belcheri* is lower than the  $10^{-4}$  M reported for *C. iredalei* (Leu 1990), *C. gigas* and *C. virginica* (Coon et al. 1985, 1986) as well as *C. lugubris* and *Saccostrea commercialis* (Jarayabhand, pers. comm.). However, the optimum concentration of NE ( $10^{-5}$  M) for *C. belcheri* is comparable to those reported for *C. lugubris* and *S. commercialis* (Jarayabhand, pers. comm.) though lower than

TABLE 1.

Summary of statistical analysis on mean setting percentage when larvae were exposed to different concentrations of the test compounds for 1 hour.

Concentration (M) Treatment	Percentage Setting (%) ( $m \pm s.d.$ )					Control
	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$10^{-3}$	
EPI	$26.7 \pm 3.5^{b,c}$	$74.0 \pm 4.5^a$	$87.0 \pm 5.3$	$74.4 \pm 1.2^a$	$32.8 \pm 5.1^b$	$24.5 \pm 1.5^{c,e,f,j}$
NE	$30.1 \pm 5.7^c$	$64.3 \pm 2.6^d$	$70.6 \pm 5.8^d$	$64.4 \pm 5.5^d$	$21.8 \pm 4.3^f$	
L-DOPA	$32.4 \pm 6.7^b$	$63.7 \pm 6.5^e$	$71.4 \pm 5.9^e$	$31.6 \pm 2.3^b$	$11.3 \pm 3.1$	
GABA	$29.5 \pm 9.1^l$	$66.9 \pm 11.1^l$	$71.8 \pm 2.3^l$	$71.8 \pm 3.4^l$	$29.8 \pm 5.7^l$	

Data sets bearing the same letters are not significantly different ( $P < 0.005$ ).

TABLE 2.

Summary of statistical analysis on mean setting percentage when larvae were exposed to different concentrations of the test compounds for 24 hours.

Concentration (M) Treatment	Percentage Setting (%) (m ± s.d.)					
	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>	Control
EPI	31.9 ± 1.8 <sup>a</sup>	61.8 ± 9.9	36.4 ± 6.7 <sup>a</sup>	4.3 ± 2.0	0.9 ± 0.1	24.5 ± 1.5 <sup>c,h</sup>
NE	35.7 ± 4.3 <sup>c</sup>	52.4 ± 3.3 <sup>b</sup>	44.7 ± 6.7 <sup>b,c</sup>	10.1 ± 1.1	3.7 ± 1.0	
L-DOPA	21.9 ± 5.4 <sup>c</sup>	56.0 ± 9.8 <sup>d</sup>	54.4 ± 6.0 <sup>d</sup>	23.9 ± 5.4 <sup>c</sup>	9.4 ± 2.3	
GABA	26.1 ± 5.6 <sup>g,h</sup>	58.1 ± 5.5 <sup>f</sup>	54.4 ± 3.1 <sup>f</sup>	49.8 ± 6.1 <sup>f</sup>	35.7 ± 4.8 <sup>e</sup>	

Data sets bearing the same letters are not significantly different (P < 0.005).

the 10<sup>-4</sup> M reported for *C. iredalei* (Leu, 1990), *C. gigas* and *C. virginica* (Coon et al. 1985, 1986).

Prolonged exposure to EPI and NE at concentrations higher than 10<sup>-5</sup> M resulted in reduced metamorphic induction, reduced

rates of morphogenetic differentiation and increased mortality. Similar observations were reported for *C. iredalei* (Leu 1990), *C. lugubris* and *S. commercialis* (Jarayabhand, pers. comm.). These observations differ from those of Coon et al. (1985, 1986) who

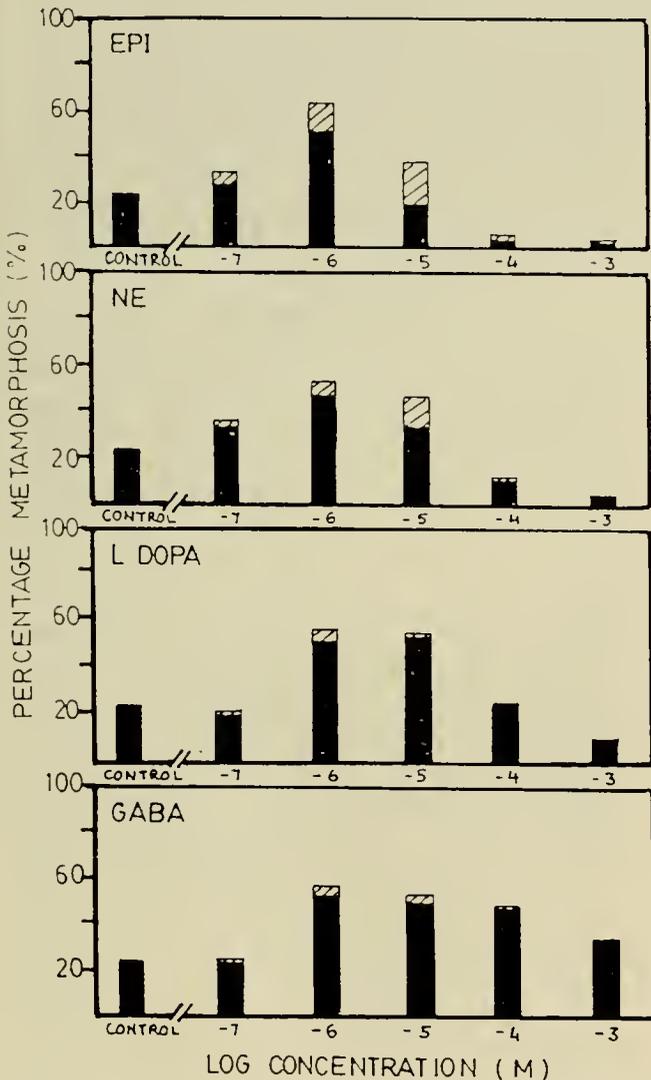


Figure 2. The relative proportion of *Crassostrea belcheri* spat which were cemented (■) and free (▨) in response to a 1 hour exposure to various concentrations of test compounds.

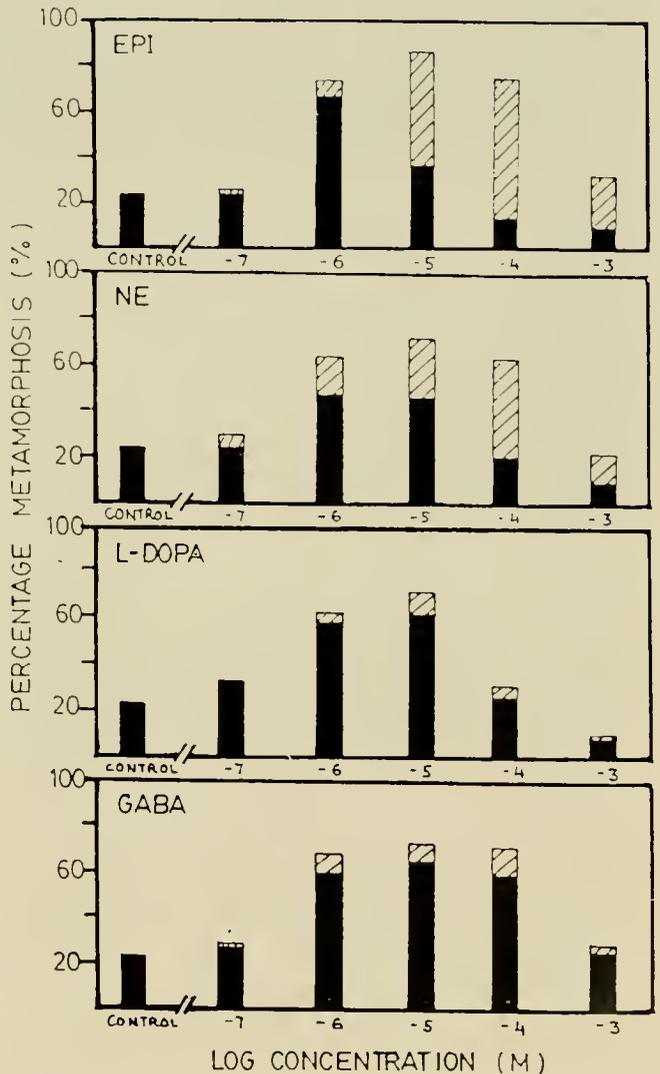


Figure 3. The relative proportion of *Crassostrea belcheri* spat which were cemented (■) and free (▨) in response to 24 hour exposure to various concentrations of test compounds.

reported that in *C. gigas* prolonged exposure to EPI and NE at  $10^{-4}$  M resulted in increased percentage set from 80 to 90% and 50 to 80%, respectively.

The percentage of free spat resulting from EPI and NE treatment (10–80%) was comparable to that reported for *C. gigas* (85–90%) and *C. virginica* (65%) (Coon et al. 1986). However, only 38.7% of *C. iredalei* spat was reported as cultchless by Leu (1990). According to Coon et al. (1986), the mechanism through which EPI and NE are able to induce metamorphosis independent of settlement behaviour is mediated through vertebrate type alpha-adrenergic receptors. Because EPI triggers metamorphosis without the settlement behaviours that normally preceded it, these receptors presumably function later, or on a different pathway in the chain of events that comprise settlement and metamorphosis, than the events that culminate in attachment.

Setting performance of *C. belcheri* eyed larvae can also be enhanced via a 1-hour exposure to L-DOPA at  $10^{-4}$  to  $10^{-7}$  M with highest setting percentage at  $10^{-5}$  and  $10^{-6}$  M. Prolonged exposure to L-DOPA at  $10^{-3}$  M resulted in a lowering of the percentage set in *C. belcheri*. In general, the response of *C. belcheri* eyed larvae to L-DOPA paralleled those reported for *C. lugubris* and *S. commercialis* (Jarayabhand, pers. comm.), *C. gigas* (Cooper 1983, Coon et al. 1985) and *C. iredalei* (Leu 1990). However, L-DOPA was not effective in inducing metamorphosis in *Chlamys hastata* (Hodgson and Bourne 1988). The same authors reported that when higher concentrations of the drug were tested ( $10^{-4}$  and  $10^{-5}$  M) they were found to be toxic to the larvae. Prolonged exposure to L-DOPA caused complete withdrawal of all tissues into the larval shell. Thus, even when L-DOPA induced metamorphosis, prolonged continuous exposure to the drug reduced the rate of morphogenetic differentiation.

The mechanism of induction as well as a model of natural settlement and metamorphosis has been proposed by Coon et al. (1985) based on the observations of the response of *C. gigas* to EPI, NE and L-DOPA. Since L-DOPA induces behaviours exhibited by the larvae in response to natural environmental stimuli and results in a complete response including both settlement and metamorphosis, L-DOPA or some L-DOPA-mimetic molecules could be sufficient environmental stimuli to initiate settlement. How-

ever, there is presently no evidence that L-DOPA, EPI or NE occurs in the  $10^{-4}$  to  $10^{-5}$  M range in nature.

The results presented here also show that GABA is effective in inducing *C. belcheri* larvae to set and metamorphose. Larvae exposed to  $10^{-4}$  to  $10^{-6}$  M GABA showed higher setting percentages (70–71%) compared to the controls (24.5%); whereas there was no significant difference at  $10^{-3}$  and  $10^{-7}$  M compared to controls. The setting performance of *C. belcheri* eyed larvae exposed to  $10^{-3}$  M GABA for 24 hours was enhanced. This may be explained according to the hypothesis proposed by Morse et al. (1980), where metamorphic responses of the abalone larvae, *Haliotis* sp. to GABA was slow due to the lack of suitable receptors. Unlike EPI, NE and L-DOPA, GABA did not exhibit toxic effects on *C. belcheri* larvae during prolonged exposure (24 hours). However, GABA was reported to be ineffective in causing bivalve larvae such as *C. gigas* (Coon et al. 1985), *C. hastata* (Hodgson and Bourne, 1988), *Perna viridis* (Baylon 1988) and *C. iredalei* (Leu 1990) to set or metamorphose.

Though post-test survival was not studied here, Coon et al. (1985) and Shpigel et al. (1989) have demonstrated that EPI induced spat of *C. gigas* and *O. edulis*, respectively, had appeared completely normal after 3 weeks to 8 months growth.

The reasons for the differences between *C. belcheri* and *C. gigas* and *C. virginica* as well as other tropical species such as *C. iredalei*, *C. lugubris* and *S. commercialis* in both the magnitude and consistency of their response to the neuroactive compounds (EPI, NE, L-DOPA and GABA) are not clear. Further research is needed to determine whether the differences in the induction of metamorphosis of bivalve larvae by neuroactive compounds is governed by species-specific factors.

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## THE STATUS AND LONG-TERM TRENDS OF OYSTER REEFS IN GALVESTON BAY, TEXAS

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**ABSTRACT** The oyster reefs of Galveston Bay were surveyed using a new acoustic technique which permitted conducting a detailed survey over a wide geographic area. The results of this survey were compared to a more limited survey conducted circa 1970 and to earlier, less detailed accounts. Our survey approximately doubles the known area of reef and unconsolidated shelly substrate in the bay system.

Certain components of the Galveston Bay reef system have persisted throughout recorded time; others have exhibited substantial malleability, changing position and shape in response to natural and man-made changes in the bay system. Regional subsidence has resulted in an increase in water depth over the reefs, thus reducing the acreage intertidally and subaerially exposed, and areas of high subsidence have suffered reef attrition due to siltation. Channelization, dike construction, and the disappearance of major barrier reef have substantially changed bay circulation patterns. As a consequence, the equilibrium that once existed between the reefs and bay circulation has been modified, resulting in loss or attrition of a number of reefs in areas of lowered salinity and the accretion of reefs parallel to the new isohaline structure of the bay. Thus, some reefs are no longer optimally located for continued high productivity; many areas of low reef coverage would now support productive reef if substrate became available. In optimal areas, reefs more than doubled in size in 20 yr.

No evidence was found to suggest that the oyster fishery had contributed to reef attrition, however leasing may have encouraged reef accretion. Artificial reefs did no worse or better than their natural nearby counterparts, indicating siting as the overriding concern for successful artificial reef emplacement. The Houston Ship Channel has extended the isohalines up estuary to the great benefit of oyster populations and the oyster fishery. Over 1000 hectares of reef have developed along this channel, a substantial fraction of which exists between the channel edge and the crest of the parallel-trending spoil banks. Both a channel and a spoil bank are required for development of reef along channels. Channels in which spoil was placed on only one side always have reef development predominately or exclusively on that side.

**KEY WORDS:** Oyster reef, bathymetry, Galveston Bay, oyster fishery, channelization, artificial reef, geographic survey

### INTRODUCTION

Oyster reefs are one of the primary geological features of most estuaries along the East and Gulf of Mexico coasts of the United States. They affect current flow and salinity and provide a primary substrate for a wide variety of hard-bottom invertebrates and fish. They support an important commercial fishery and are utilized extensively for recreational harvest (Broutman and Leonard 1988). Accordingly, oyster reefs play a pivotal geological, ecological and commercial role in estuaries and knowing their status and long-term trends has become an important component of estuarine management (e.g., Haven and Whitcomb 1983; May, 1971). However, the oyster reefs of most bays have either never been surveyed or surveyed only once. Thus, our understanding of long-term trends is based primarily on comparison to anecdotal evidence.

Galveston Bay epitomizes most of these general truths. The commercial oyster fishery in Galveston Bay is one of the more important ones in the U.S., and the private (or noncommercial) harvest of shellfish ranks Texas third in the country (Hofstetter 1990, NOAA 1991a,b). Concern about the condition of the oyster reefs in Galveston Bay is one of long-standing (TGFOC 1929, Eckhardt 1969, Benefield 1976). However, prior to the late 1960s, few quantitative data were available despite the earlier surveys in many of the central Texas bays (Moore 1907, Moore and Dangle 1915) and elsewhere in the Gulf of Mexico (e.g., DeAlteris 1988). In the late 1960s, the Texas Parks and Wildlife Department began mapping the oyster reefs of Galveston Bay, primarily in response to concerns about the extent of shell dredging (shell mining for road base, for instance) in the bay (Benefield and Hofstetter 1976) and to map the commercially important oyster

reefs. This survey was completed in the early 1970s. Since then, little additional information has been collected.

The purpose of this study was to survey the oyster reefs of Galveston Bay and compare them to the earlier surveys to quantitatively characterize the long-term trends in reef coverage. Of particular importance were concerns about the perceived loss of reef area and the lowering of relief on the remaining reefs as a result of the oyster fishery. Continual use of the 1 to 1.3 m dredges used by the fishery, it was feared, might wear down the reefs, and continual shell removal without replenishment might eventually destroy them. Of equal concern was the impact of channelization on bay oyster production, the influence of subsidence and siltation on reef coverage, and the determination of the degree of recovery from shell dredging, an activity that was prohibited in Galveston Bay after the early 1970s. Accordingly, a complete survey of the oyster reefs of Galveston Bay began in 1991, the results of which are reported here.

### METHODS

#### *Discrimination of Bottom Type*

A dual frequency acoustic profiler consisting of a Datasonics Dual Frequency Transceiver (Model DFT-210), a Datasonics towed fish with dual transducers (27 and 300 kHz) and an EPC Multichannel Chart Recorder (Model 4800) was used to discriminate bottom type. The technique was described by Simons et al. (1992). Primary identification of oyster reefs relied on the record from a 300 kHz channel. On the chart paper, an oyster reef appears as a dark, dense series of spikes projecting well above the background signature from a mud or sand bottom (Figures 1–4 in Simons et al. 1992). DeAlteris (1988) noticed a similar signature with a 200 kHz transducer; however, the reliability of the signature was not satisfactory. He relied on a second echo from the hard

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bottom. The 300 kHz signature is unambiguous. Although we have not investigated the acoustic phenomena involved, we surmise that the oyster reef signature results from more sound energy bouncing back to the transducer. In the case of a muddy bottom, more of the sound energy is absorbed, thus the signature is reduced. Sand and shell hash give an intermediate, fuzzy signature, still readily distinguishable from reef or other oyster bottom.

In practice, we encountered only 2 bottom types that required occasional groundtruthing to verify their non-oyster nature: clam beds and coarse shell hash usually associated with points, near-shore sediments and dredge spoil. With experience, coarse shell hash could be discriminated with relative ease and required little groundtruthing. With experience, most clam beds could also be distinguished; however, dense clam beds required groundtruthing. The acoustic technique, then, could be used to identify concentrations of most large epifaunal or semi-epifaunal shellfish, not just oysters.

A 27 kHz record was used to discriminate reefs from oysters on muddy bottom or spoil. We distinguished 3 bottom types; sandy or muddy bottom, oyster reef, and unconsolidated shelly sediment. The distinction between reef and unconsolidated shelly sediment was somewhat arbitrary as was the distinction between the latter and sandy or muddy (non-shelly) bottom. In general, oyster reef contained a hard substrate in the immediate subsurface. Unconsolidated shelly sediment did not. Occasional groundtruthing confirmed the general accuracy of this distinction; however, every individual case was not verified. Small reefs and towheads [Hill and Masch (1969) define a towhead as a reef of 10 acres or less] frequently were too small to generate a reef-like subsurface signal although clearly reefal in nature and the substrate under points (e.g., Red Bluff or Dollar Point in Galveston Bay, Fig. 1) (see insert for Fig. 1) frequently yielded a strong reef-like return presumably due to the relatively hard basement material forming the point and the meager amount of sediment accumulated upon it. Consequently the reader is cautioned to consider the general distribution of these bottom types as more accurate than the designation at any specified location.

The acoustic apparatus was used throughout with 2 exceptions. First, certain shallow reefs in Galveston Bay proper (e.g., Todd's Dump) were too shallow to be mapped. In these cases, we ran lines up to these reefs on both sides and visually determined substrate in-between. Estimation of areal extent was not compromised by this method. Secondly, in the Deer Islands area and the Christmas Bay sector of West Bay (including Bastrop Bay, Chocolate Bay and Oyster Lake), the poling method was used because extensive areas of shallow water prohibited the use of the acoustic array. We ran calibration lines using both methods. Reef (hard substrate) was equivalently identified by both techniques. Unconsolidated shelly sediment, however, was occasionally not equivalently distinguished from sand and mud. The calibration lines allowed us to correct for this bias. The position of the lines run are shown in Figures 2 and 3.

#### *Determination of Position and Relief*

Position was determined while underway using a Magellan Global Positioning System (GPS). A differential Coast Guard beacon receiver was used in 1993 to maintain precision when the standard GPS signal was degraded by the U.S. Department of Defense. A GPS system was necessary for accurate determination of position. Many reefs were less than 20 m across in shortest

dimension and larger reefs had significant variations in relief of a similar scale. In practice, the precision of navigation was within 0.01 min latitude and longitude on all days.

Relief was recorded while underway using an Apelco fathometer during profiling or by direct measurement when poling. Pictures of the fathometer screen were taken with a 35 mm camera to record relief of all reefal area because relief changed too quickly to be recorded manually while underway. Fathometer accuracy declined at depths <0.8 m. Extensive shallow areas were surveyed by pole to maintain bathymetric accuracy.

#### *Procedure for Data Collection*

The chart recorder was continually monitored and annotated with position and depth information at least once every minute. When reefs or rapid changes in bathymetry were encountered, positions and depths were recorded at more frequent intervals. Further details of reef relief and position were taken from the pictures of the fathometer screen and calibrated with the chart recorder knowing the speed of the chart paper and the fathometer screen.

While poling, substrate type, depth and position were recorded no less frequently than 0.05 min latitude or longitude. Although the acoustic survey provided a continuous record, the data used for production of the accompanying maps utilized substrate type and position data obtained from the continuous survey in approximately the same intervals of latitude and longitude as obtained by poling so that the distribution of substrate depicted on the maps was based on similar data qualitatively and quantitatively for the 2 survey methods.

For data collection, N-S and E-W lines were run on an 0.25 min grid to map uncharted areas. Subsections having reefal components were then mapped using an 0.125 min grid. The grid choice was a compromise between the detail required to adequately assess reef coverage, the accuracy of navigation, and the time required to run the lines.

#### *Laboratory Analysis and Data Processing*

Because depth changed during the day and from day to day with the tides and wind setup, the bathymetric data were standardized to a constant datum. To do so, we extended the 0.125 min grid used in data collection beyond the reef boundaries out over areas of relatively deep, flat muddy bottom so that each line and the intersection of several N-S and E-W lines occurred in areas where the depth record was most accurate and where depth changes were minimal. This permitted an internal standardization of depth between lines run at different times, all of which could then be corrected to mean sea level by 1 standard correction. The internal standardization involved identifying all line crossings in which depth records fell within 0.06 min of each other over non-reefal bottom and in areas where depth changed by less than 0.15 m in 0.125 min. The median depth difference for all such cases for any one line was used to determine the correction for that line to the internally standardized depth. In practice, about 90% of the lines could be corrected automatically by computer using this approach. A few lines, which perforce by their location extended nearly exclusively over reef or over areas of substantial depth change, were corrected by hand to the internally standardized

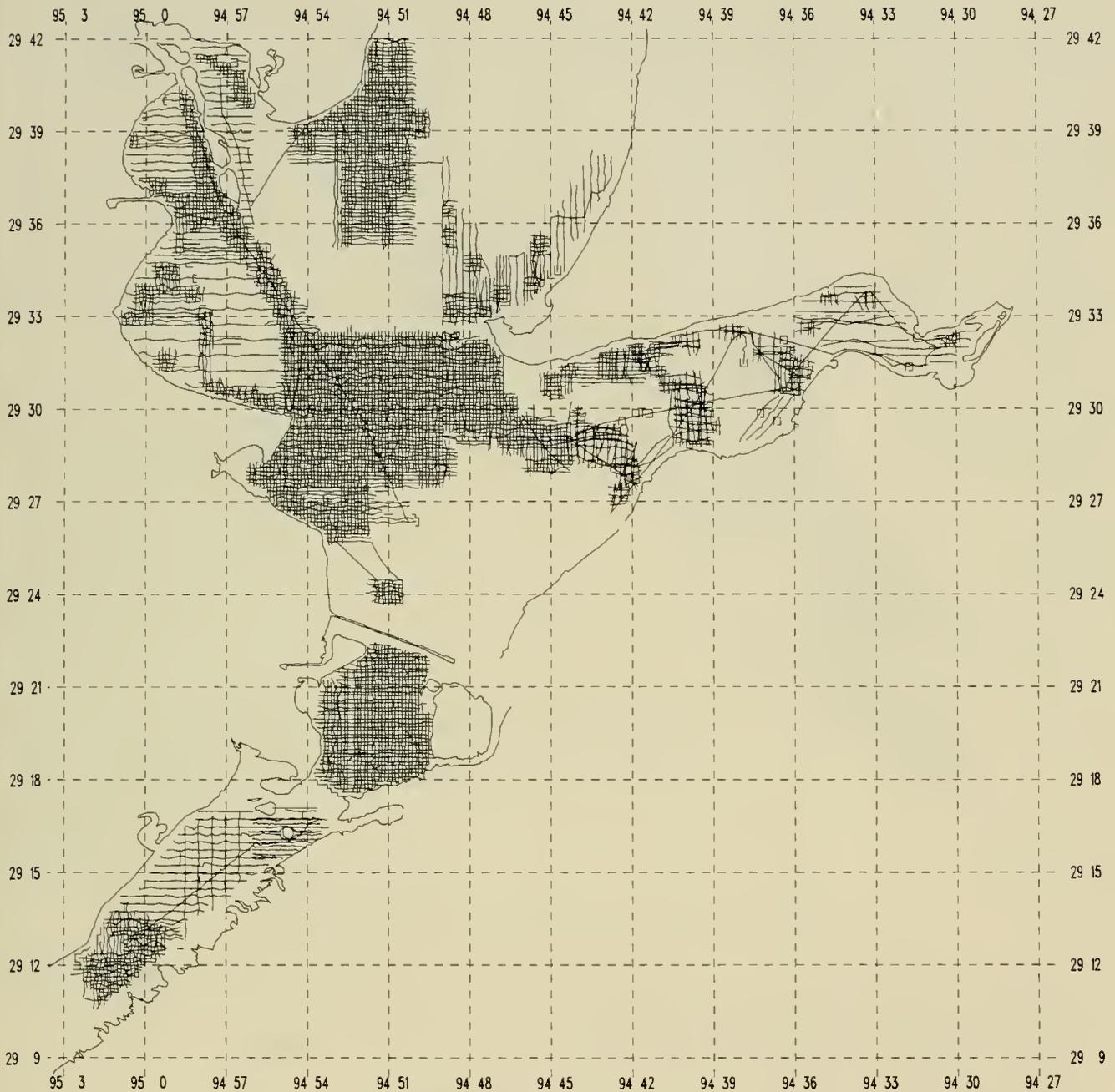


Figure 2. The transect lines run during the oyster reef survey in Galveston Bay proper.

depth. The Army Corps of Engineers tide staff at Eagle Point was used to calibrate the bathymetry to mean sea level.

Once the depth corrections were completed, Arc/Info, a Geographic Information System, was used to estimate reef area and bathymetric detail. The subroutine library TINS was used to form Thiessen polygons from the spatial array of latitudes and longitudes and substrate designations. Reef areas were calculated by summing the areas of the appropriate polygons.

In many areas near shore and in extensive areas in West Bay, the quantity of shell on the sediment surface gradually declined rather than stopping abruptly. In these areas, it was necessary to arbitrarily define the boundary between unconsolidated shelly sediment and sand or mud. In general, we took the boundary as a shell

content half the average regional high, so that sediments designated as unconsolidated shelly sediment contained substantial shell and areas designated non-shelly contained distinctly less shell. However, an oyster dredge would undoubtedly recover shell in many of these latter areas.

With a few exceptions, the grid was sufficiently fine to define the extent and shape of the reefs. Exceptions were the fields of towheads frequently encountered in oil fields and occasionally elsewhere in the bay. Undoubtedly, many small towheads were missed by the survey; however, areas where towheads were common are readily seen in Figures 4 (see insert for Fig. 4) and 5. Similarly, many small reefs along channels and associated with leases may have been missed. However, the locations where these

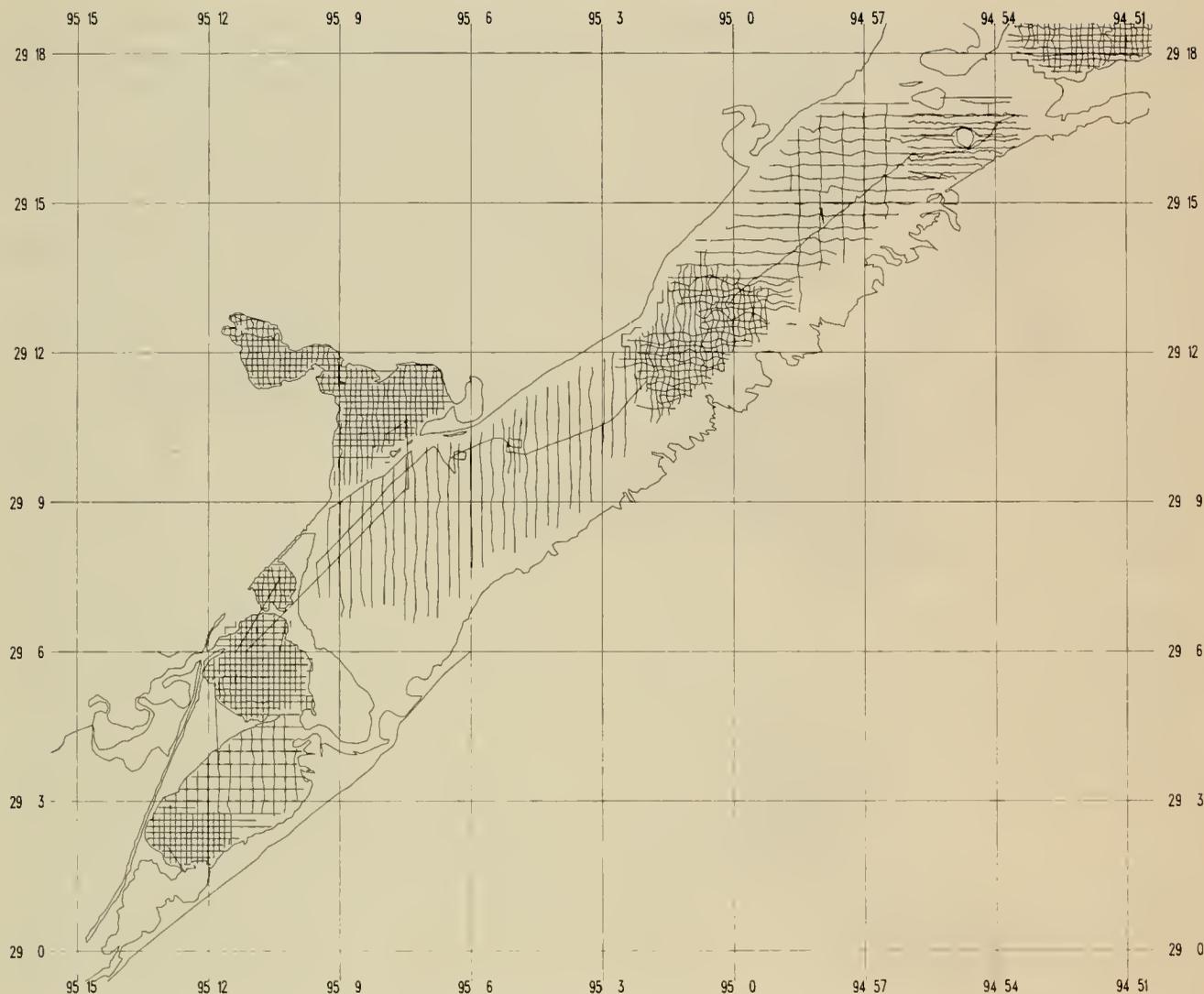


Figure 3. The transect lines run during the oyster reef survey in West Bay.

reef types are common is readily observed. Because of the statistical method used to generate the maps from a grid of transect lines, the estimated reefal coverage for these regions is more accurate than the distribution of individual towheads.

A series of 16 detailed maps,<sup>1</sup> each covering a 6-min latitudinal by 6 or 9-min longitudinal area, were created covering the mapped areas of the bay (Powell et al. 1993a,b), the distillation of which appears as Figures 4 and 5. In some cases, in determining the area of reefs, the resulting area summed from the Thiessen polygons was equivalent to that generally associated with a known reef. San Leon Reef is an example. In other cases, nearby towheads or small reefs were included with the primary reef rather than erecting a large number of new names for minor reefs. Old Yellow Reef was an example. In a few cases, reef accretion resulted in the loss of the discreteness of a previously named reef. In some cases, we attempted to estimate the reef area (e.g., Archie's Reef). In other cases, we simply dropped the name from the maps. Bathymetric detail, a more complete explanation of the methods used in map

production, and details concerning the areal measurement of individual reefs can be found in Powell et al. (1993a,b).

## RESULTS

### Reef Description

#### Overview and Perspective

Figure 1 gives a synopsis of the names of the topographic and other features referred to in this report. Figures 6 and 7 (see insert for Figs. 6 and 7) provide the locations of each named reef and Figure 8 depicts the areas of greatest reef gain and loss. Table 1 gives the areas of the individual reefs. Reef and unconsolidated shelly sediments comprised a total of 10794.5 hectares of the surveyed bay area. Of this, about 53.3% (5754 hectares) was in Galveston Bay, East Bay and Trinity Bay (Table 2). The remaining 46.7% was in West Bay and the Pelican Island Embayment. (The term embayment refers to sectors of Galveston Bay proper separated by significant points, islands, or man-made dikes and channels. For example, the Clear Lake Embayment is that portion of the bay between Eagle Point and Red Bluff out to the Houston

<sup>1</sup>The detailed maps can be ordered from the first author.



Figure 1. Cartoon of the Galveston Bay system showing important geographic features and physical characteristics and the areas of significant reef accretion and lo-



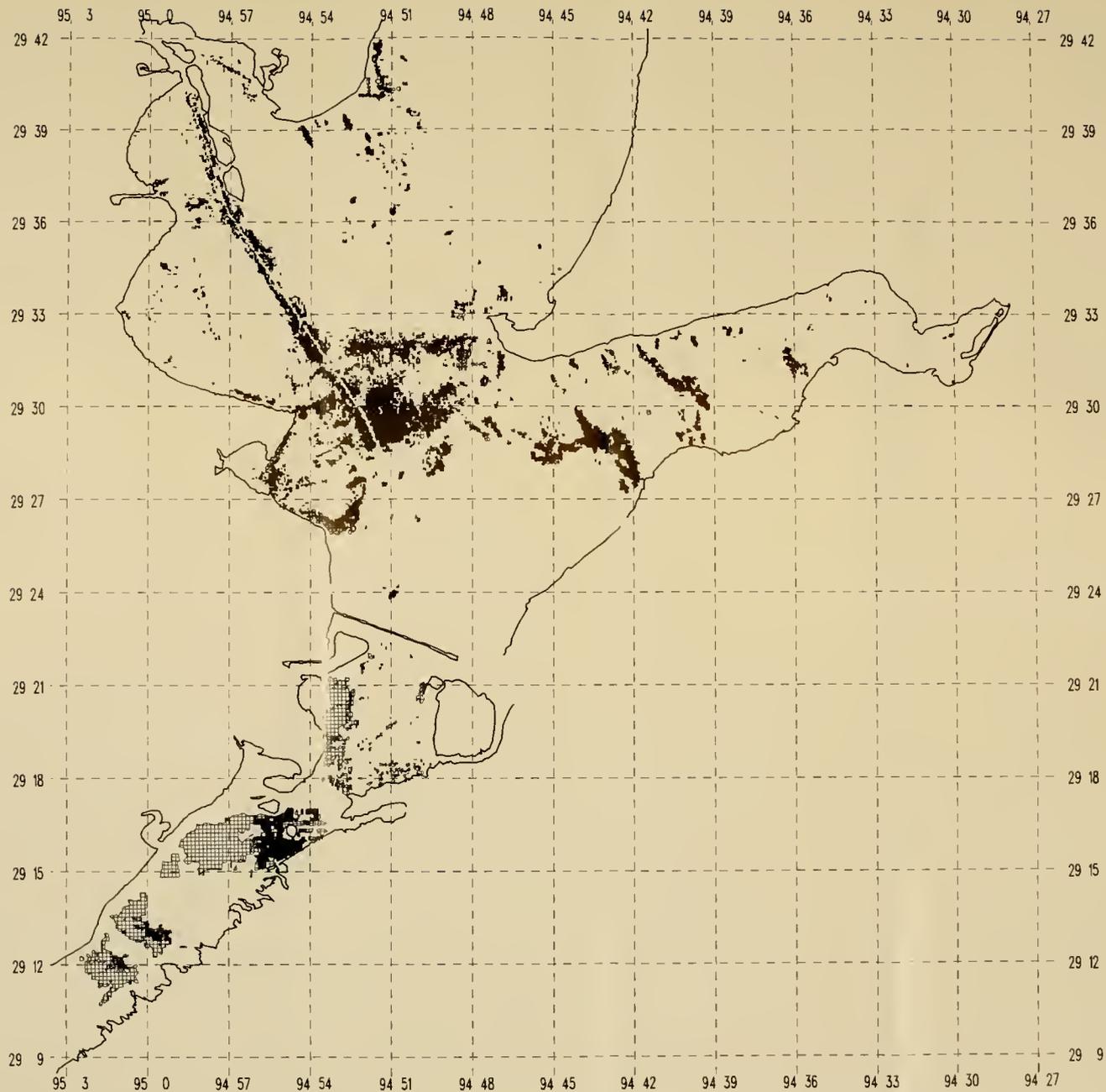


Figure 4. The oyster reefs of Galveston Bay proper. Key to abbreviations and reef areas listed in Table 1. Reefs identified in Figure 6. Solid, oyster reef; hatched, unconsolidated shelly sediment.









Figure 7. The location of reefs in West Bay and its satellite bays identified by their abbreviations as translated in Table 1 and the location of important geographic features in West Bay and its satellite bays.



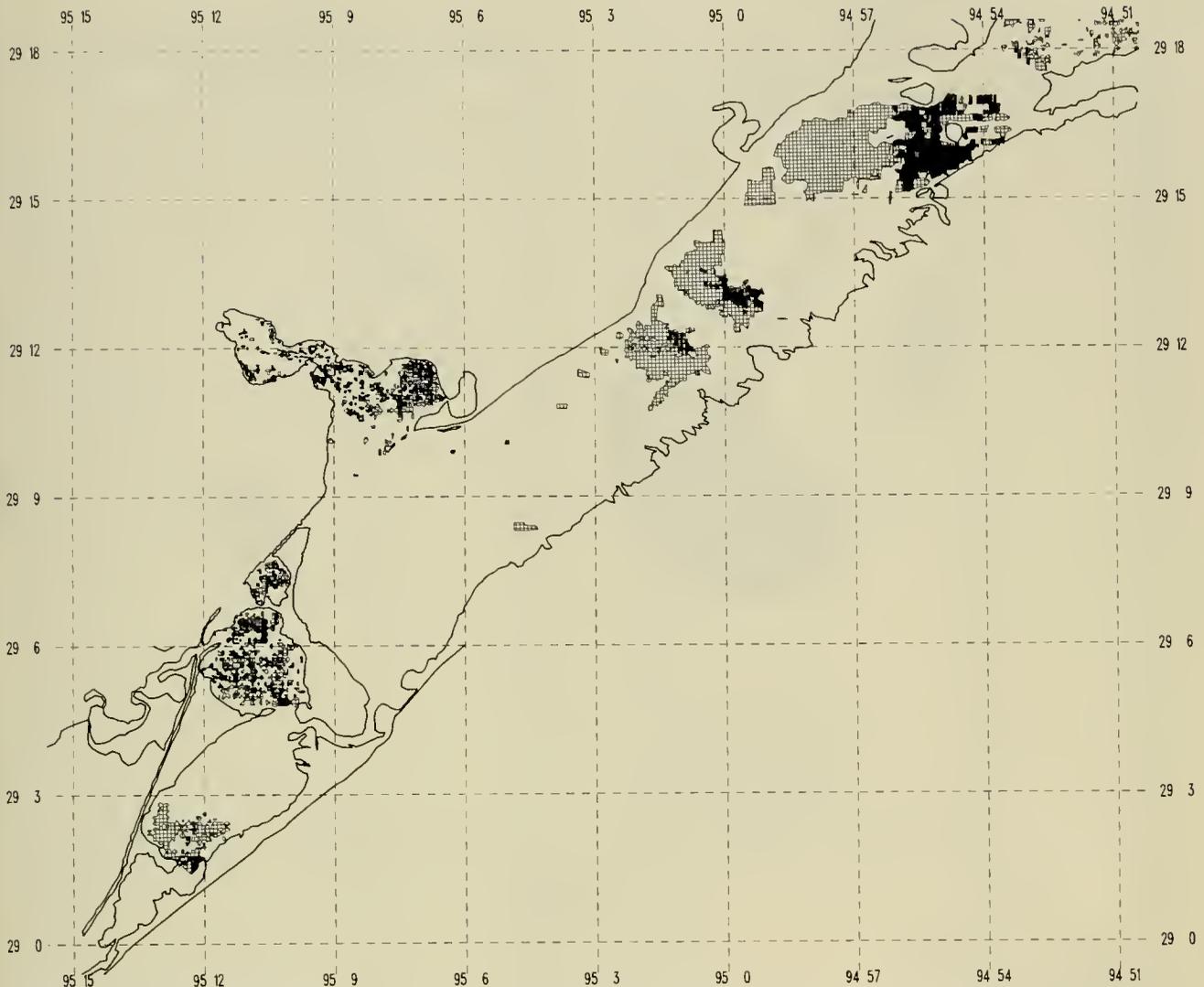


Figure 5. The oyster reefs of West Bay. Key to abbreviations and reef areas listed in Table 1. Reefs identified in Figure 7. Solid, oyster reef; hatched, unconsolidated shelly sediment.

Ship Channel. The Dickinson Embayment is that portion between the Texas City Dike and Eagle Point out to the Houston Ship Channel) (Fig. 1).

The Galveston Bay system was subdivided into 11 sectors (Table 3). The majority of the reef and unconsolidated shelly sediments of the bay was located in East Bay, on and north of Redfish Bar in central Galveston Bay, in the Dickinson Embayment, along the Houston Ship Channel, and in West Bay and its satellite bays. Trinity Bay, the Red Bluff/Morgan Point Embayment and the Clear Lake Embayment contributed only 6.8% of the bay-system total and only 12.8% excluding West Bay and the Pelican Island Embayment. West Bay contributed 27.7% of the total and its satellite bays another 11.2%.

#### Natural Reef

The reefs of the Galveston Bay system were divided into those primarily of natural origin and those primarily of anthropogenic origin. Natural reef was of 5 distinctive types.

(1) Barrier reefs extended significant distances across the bay. Typically, these reef tracts ran perpendicular to the prevailing shoreline. Examples include Carancahua Reef and the Confederate/North and South Deer Island Reef complex in West Bay, the Drum Village/Gale's/Middle Reef complex in East Bay, the Hanna Reef complex in East and Galveston Bays, the Todd's Dump/Redfish Bar complex in Galveston Bay, Arcadia and Christmas Point Reefs in Christmas Bay, and a series of reefs in Chocolate Bay off, for example, Shell Point and Horse Grove Point.

(2) Smaller reefs extended perpendicular from shore throughout the Galveston Bay system. Examples include Dow and Big Beezley Reefs in Trinity Bay, Stephenson and Moody Reefs in East Bay, and Dollar and Red Bluff Reefs in Galveston Bay. Most of these reefs were detached from the shoreline. The only exceptions were a few reefs in East Bay such as Richard's Reef. Many, but not all, of these reefs were associated with points suggesting an underlying geological control. Most oyster reefs begin on local topographic highs, whether natural or man-made.

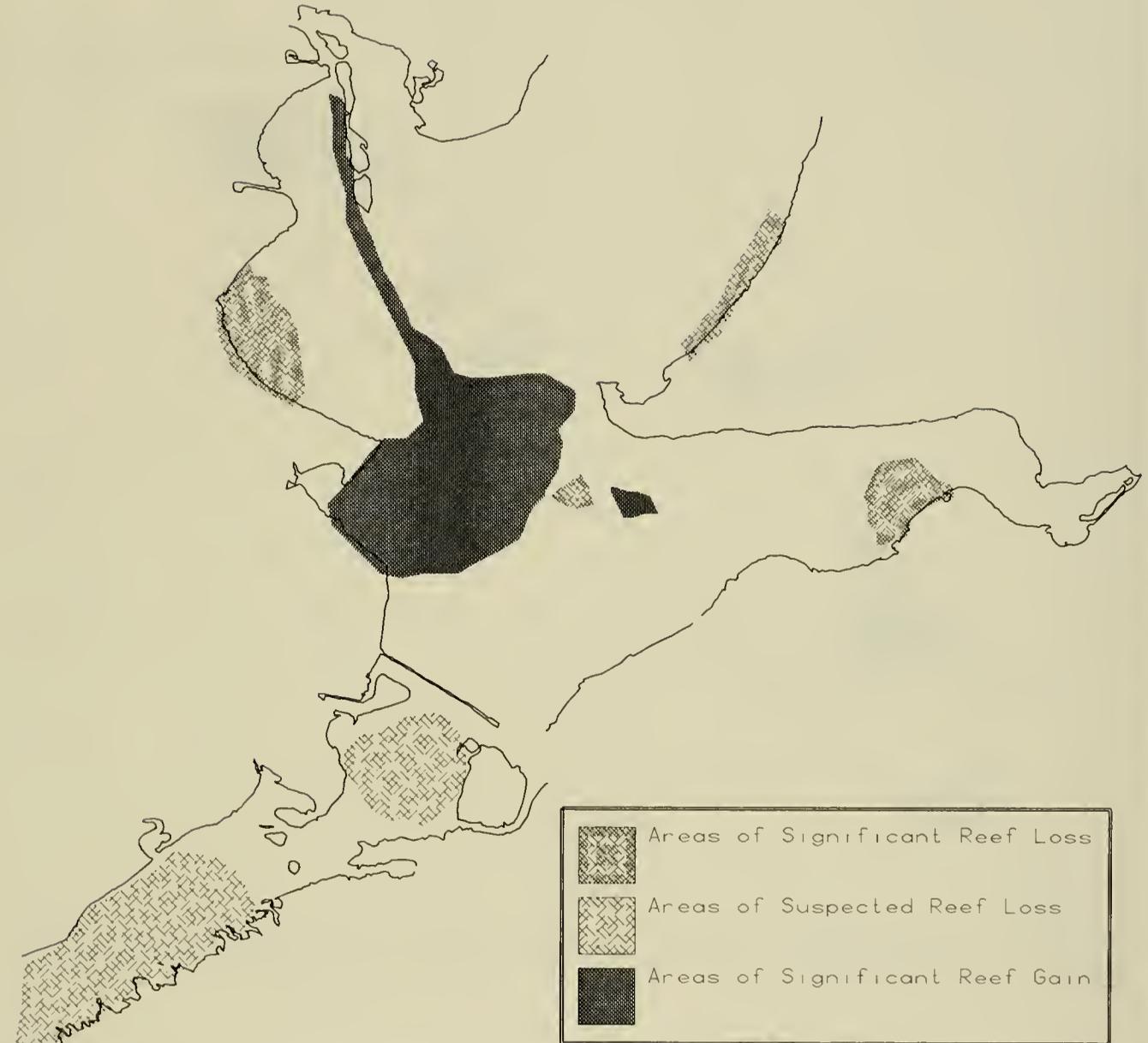


Figure 8. Distribution of areas recording the greatest rates of reef accretion and attrition over the 20+ yr period separating the circa-1970 survey and the present survey.

(3) Alongshore reefs, like Levee Reef in Galveston Bay and Elliotts Reef in Trinity Bay, probably follow drowned beach lines. These reefs, typically, are also detached from the present shoreline. April Fools Reef is the significant exception. Most of these reefs also contain significant fractions of unconsolidated shelly mud and sand as well as consolidated reef. Alongshore reefs were also common in the satellite bays of West Bay (e.g., Chocolate Bay, Oyster Lake and Bastrop Bay), but these could not be attributed to the same causative mechanism. Many of these latter reefs had little or no relief.

(4) Patch reefs and towheads were small to medium-size reefs roughly circular or irregularly elliptical in outline. This reef type was most common as it formed a discontinuous line across the mouth of Trinity Bay, along the northern and southern shorelines of East Bay, and within the major oil fields and leased areas of the bay (Anonymous 1988, Hofstetter 1990).

(5) Expanses of low-relief unconsolidated shelly mud were surveyed in West Bay and the Pelican Island Embayment. This bottom type was uncommon elsewhere in the Galveston Bay system.

#### Anthropogenic Reef

We attempted to estimate the amount of reef purposefully created by man or originating as a result of man's activities in the bay. Overall, anthropogenic reef, as a rough approximation, contributed about 18% of the reef in the Galveston Bay system. Anthropogenic reef was of 4 types.

(1) Most oyster leases contained reef. Some leases were clearly located on preexisting natural reef. Elsewhere, whether lease-associated reef originated naturally or from shell planting, could not easily be discerned.

TABLE 1.

The areas of individual reefs in the Galveston Bay system and their abbreviations.

Reef	Abbreviation	Area (ha)
Anahuac Reef	AH	2.120
Anderson Reef	AN	7.509
April Fools Reef	AF	43.214
Arcadia Reef	AR	53.322
Arcadia Shell	AS	194.799
Archie's Reef	AR	3.426
Bar 24 Reef	B24	2.226
Bart's Pass Reef	BRP	68.956
Bart's Pass West	BRPW	81.325
Bastrop Bayou Reefs	BB	99.284
Bayport Channel Reefs	BPC	10.349
Bayview Reef	BV	6.302
Bent Pipe Reef	BP	12.859
Big Beezley Reef	BB	30.927
Black Sheep Reef	BLSP	112.464
Bob's Knob	BK	2.030
Buckshot Reef	BKS	8.852
Bull Hill	BH	114.052
Bull Shoals	BLSS	40.264
"C" Reef	CC	5.432
Carancahua Reef	CA	542.919
Catfish Reef	CT	9.935
Cedar Bayou Channel Reefs	CBC	28.855
Central Clear Lake Reefs	CCL	8.565
Chocolate Bay Channel Reefs	CB	105.136
Christmas Point Reef	CP	29.316
Clamshell Reef	CSL	4.330
Clear Lake Channel Reefs	CLC	8.237
Confederate Reef	CF	317.554
County Line Reefs	CL	31.585
Courthouse Reef	CH	1.953
Cowshed Reef	CSD	14.758
Crescent Reef	CR	3.621
Cross-island Reef	CI	69.776
Crow's Nest Reef	CN	1.840
Deer Island Shell	DIS	1032.437
Dee's Reef	DE	8.174
Desperation Reef	DP	29.852
Dickinson Reef	DKN	64.028
Dickinson Channel Reefs	DKC	40.330
Dollar Reef	DL	215.848
Dow Reef	DW	40.830
Drum Village Reef	DV	37.508
Dryhole Reef	DH	5.746
East Redfish Reef	ERF	82.014
East Red Bluff Reef	ERB	3.377
Eagle Point Reef	EP	14.725
Eddy Reefs	ED	6.849
Elliotts Reef	EL	39.789
Experimental Reef	EX	2.344
Fisher Reef	FS	123.757
Found Reefs	FD	10.354
Four Bit Reef	FB	9.206
Frenchy's Reef	FR	74.879
Gale's Reef	GL	95.578
Galveston Shell	GV	80.481
Gaspipe Reef	GP	20.687
Grassy Point Reefs	GP	14.823
Green's Cut Shell	GC	114.987

TABLE 1.

continued

Reef	Abbreviation	Area (ha)
Half Moon Reef	HM	16.717
Halfway Reef	HW	10.778
Halls Embayment Reefs	HE	198.086
Hanna Reef	HN	475.067
Hodge's Reef	HD	3.645
Horse Grove Point Reefs	HG	36.814
Humble Reef	HB	.606
I-45 Shell and Reefs	I45	53.870
ICWW Shell	IC	33.289
Intracoastal Reefs	IN	5.454
Island Reef	IS	3.689
Levee Reef	LV	81.109
Little Beezley Reef	LLZ	6.066
Little Bird Reef	LBD	.349
Little Half Moon Reef	LHM	1.348
Little Hanna Reef	LHN	28.425
Little Scott Reef	LST	3.130
Little Vingt-et-un Reef	LVN	1.875
Lonesome Reef	LM	8.222
Lone Tree Reef	LT	8.288
Lonely Crescent Reefs	LC	29.482
Lost Reef	LS	10.831
Lost Beezley Reef	LTBZ	56.890
Lower West Bay Reefs	LWR	4.292
Lower West Bay Shell	LWS	43.499
Marsh Reef	MH	10.158
Mary's Reef	MY	18.414
Mattie B. Reef	MTB	14.938
Middle Reef	MD	110.280
Middle Beezley Reef	MBZ	12.075
Missing Reef	MS	9.175
Moody Reef	MO	15.900
Morgan Point Reefs	MP	14.122
Moses Gate Reef	MG	6.262
Northbar Reefs	NB	142.879
North Bull Hill Reef	NBH	59.160
North Deer Island Reefs	NDI	190.811
North Redfish Reef	NRF	237.984
Northshore Reefs	NS	1.077
Nymph Point Reefs	NP	41.829
Old Yellow Reefs	OY	19.976
Outer Beezley Reef	OBZ	20.674
Oyster Lake Reefs	OL	77.321
Pasadena Reef	PD	49.853
Pelican Reef	PL	41.019
Pelican Island Shell	PI	31.476
Pepper Grove Reefs	PG	70.355
Possum Pass Reef	PP	72.181
Ray's Reef	RY	20.187
Red Bluff Reef	RB	29.979
Redfish Island	RFI	96.434
Resignation Reef	RS	34.192
Richard's Reef	RC	17.833
Roberts Reef	RT	10.884
Robinson Reefs	RN	3.799
San Leon Reef	SN	34.385
Santa Reefs	STA	7.112
Satellite Reef	STL	1.183
Scott Reef	SC	13.478
Sheldon Reef	SHN	46.164

TABLE 1.  
continued

Reef	Abbreviation	Area (ha)
Shell Island Reef	SHI	510.572
Shell Point Reefs	SP	16.256
Shoal Reef	SHL	1.974
Slim Jim Reef	SJ	31.236
Smith Reef	SMH	9.732
Smith Point Reefs	SMP	43.362
Snake Island Reefs	SNI	18.347
South Deer Island Reefs	SDI	238.153
South Redfish Reef	SRF	702.109
Spoonbill Reef	SB	6.958
Stephenson Reef	SP	10.545
Swan Lake Shell	SWL	618.234
Swirl Reefs	SW	98.915
Terry's Ridge	TYR	17.268
Tem Reef	TRN	14.906
Tidewater Reef	TW	7.230
Tin Can Reefs	TC	10.095
The Leases	TL	65.848
Todd's Dump	TD	187.288
Tom Tom Reef	TT	3.573
Tong Reefs	TNG	4.184
Triangle Reef	TRG	7.796
Trinity Reef	TRY	6.072
'Tween Reefs	TW	63.197
Upper Beezley Reefs	UBZ	7.507
Village Reefs	VI	125.248
Vingt-et-un Reef	VN	28.961
West Red Bluff Reef	WRB	5.859
Western Reefs	WE	18.710
Whitehead Reef	WH	32.399
Yacht Club Reef	YT	19.057
"45" Reefs	45	26.264
"53" Reefs	53	177.977
"59" Reefs	59	192.746
"63" Reefs	63	146.898
"75" Reefs	75	260.805
"79" Reefs	79	55.494
"85" Reefs	85	51.714

(2) Besides lease-associated reefs, a number of other reefs originated as deliberate shell plantings. Most of these reefs, termed artificial reefs (Benefield and Hofstetter 1976, Diener 1975, Hill and Masch 1969), originated as mitigation projects for shell dredging or were designed to enhance the oyster fishery (Benefield and Hofstetter 1976). A listing for the Galveston Bay system is presented by Lukins (1993). Only a rough estimate of the acreage of this reef type could be made as many sites occurred in areas

TABLE 2.

The total amount of reef and unconsolidated shelly sediments in the Galveston Bay system.

Total Reef Area	
Galveston Bay System	Galveston Bay Excluding West Bay and Pelican Island Embayment
10794.544 hectares	5754.479 hectares
41.6 square miles	22.2 square miles
about 10.4% of bay area	53.3% of total reef

occupied by natural reef so that the area estimated may not have been entirely of artificial origin. We estimate that leases and artificial reefs contributed about 1.4% of the total reef, 7.8% of the anthropogenic reef, or 175 hectares of the Galveston Bay reef system (Table 4).

(3) Oil field operations, through the emplacement of shell pads and pipe lines, accounted for significant reef development. Most oil fields contained a few to many patch reefs. In some cases, such as north of Redfish Bar, these patch reefs have coalesced to form extensive areas of shelly bottom mostly of low relief. Linearly trending sequences of patch reefs probably follow pipe line routes. Once again, naturally occurring reef probably exists in many of these areas, but could not be differentiated from anthropogenic reef, so that an estimate of reef area originating from oil field development can only be an approximation. We tentatively attribute about 375 hectares, 3.5% of the reef in the Galveston Bay system, or 19.1% of the anthropogenic reef to this mode of origin.

(4) All significant channels were lined by spoil banks that served as sites for reef development. [One of the primary requirements of reef initiation would seem to be a small (a foot or less) elevation above the surrounding bay bottom.] These channels include the Cedar Bayou Channel, the Intracoastal Waterway, the Dickinson Bay Channel, the Bayport Channel, The Chocolate Bay Channel, and the channels in Bastrop Bay. In all likelihood, little of this reef is natural, so that this fraction of anthropogenic reef is estimated more accurately than the former three. However, the acreage in Bastrop Bay was not included because it proved impossible to consistently distinguish reef on channel spoil from natural reef adjacent to the channels. Excluding Bastrop Bay, about 382 hectares, 19.3% of the anthropogenic reef, or 3.5% of the reef in the Galveston Bay system is of this origin.

(5) Besides the smaller channels, the spoil banks lining the Houston Ship Channel contributed significant reef to the bay system. This channel runs the length of Galveston Bay from the inlet, Bolivar Roads, to the Port of Houston. Our estimates do not include that portion of South Redfish Reef (the bay's largest reef) lining the ship channel and so are certainly an underestimation, probably by several hundred hectares. We estimate a minimum of 1092 hectares associated with this channel, over half of all anthropogenic reef (55.7%), and 10.1% of the entire reef area in the Galveston Bay system. Significantly, the reef along the Houston Ship Channel ranked as the third most significant single contributor to the bay's oyster shell coverage behind the barrier reef, Redfish Bar, and the expanses of low-relief unconsolidated shelly mud in West Bay.

#### Circa 1970/1991 Survey Comparison

#### Background

Although a few long-term trends can be assessed using pre-1970s navigational charts, the only quantitative comparison that can be made to the present survey is one with a circa-1970 survey (Benefield and Hofstetter 1976, Texas Parks and Wildlife Dept. 1976). Comparison of our survey with the circa-1970 survey assumes that methodology and survey coverage were similar enough to yield similar results. To this end, Benefield and Hofstetter were interviewed to obtain firsthand information about their survey to permit a more accurate 20-yr comparison.

Besides differences accruing from true reef accretion or loss over this period, a number of discrepancies between the 2 surveys

TABLE 3.

The area of surveyed reef and unconsolidated shelly sediment in each of 11 sectors in the Galveston Bay system.

Total Reef Area				
	Area (in hectares)	Area (in sq. miles)	Percent of Total Reef	Percent of Total Reef Exclusive of West Bay
East Bay <sup>3</sup>	1157.360	4.5	10.7%	20.1%
Trinity Bay <sup>3</sup>	506.146	2.0	4.7%	8.8%
Redfish Bar <sup>2,4</sup>	1336.049	5.2	12.4%	23.2%
North Redfish Bar <sup>2,4</sup>	578.038	2.2	5.4%	10.1%
Red Bluff/Morgan Point Embayment <sup>2,5</sup>	123.347	0.5	1.1%	2.1%
Clear Lake Embayment <sup>2</sup>	111.285	0.4	1.0%	1.9%
Dickinson Embayment <sup>2</sup>	850.024	3.3	7.9%	14.8%
Pelican Island Embayment	835.697	3.2	7.7%	—
West Bay	2995.224	11.6	27.7%	—
West Bay Satellite Bays <sup>6</sup>	1206.982	4.7	11.2%	—
Houston Ship Channel <sup>1</sup>	1092.230	4.2	10.1%	19.0%

<sup>1</sup> Does not include extensive acreage adjacent to the channel forming part of South Redfish Reef.

<sup>2</sup> Exclusive of the reef associated with the Houston Ship Channel except for that associated with South Redfish Reef where a delineation between channel and non-channel reef could not be made.

<sup>3</sup> Exact value depends upon the boundary defined between East Bay or Trinity Bay and Galveston Bay.

<sup>4</sup> Exact value depends upon the boundary defined between Redfish Bar and the reef system north of Redfish Bar.

<sup>5</sup> Includes the Cedar Bayou branch of Galveston Bay.

<sup>6</sup> Includes Christmas Bay, Bastrop Bay, Oyster Lake, Chocolate Bay.

originate in the limitations in technology in the circa-1970 period and in differences in the areas surveyed. The circa-1970 survey was conducted using poling to determine substrate type and sightings for position (Benefield and Hofstetter 1976). It is a credit to this survey team that many of the reefs, when compared to our survey, show only 10 to 20% differences in areal extent between the 2 techniques, despite the limitations in technology and navigation that faced the earlier surveyors. Accordingly, the 2 methods, which certainly define the edges of the reefs somewhat differently, yield qualitatively and nearly quantitatively the same results. True reef accretion or loss might, therefore, be identified with certainty.

The limitations of the poling method limited the circa-1970 survey in several ways: (1) Surveys of areas of the bay dominated by patch reefs, were limited because the running of long lines in

search of small reefs by poling was not practical; (2) Reefs in deep water (>3.3 m) were generally not surveyed. Poling in deep water was not practical and wave and current action made pole emplacement for sighting difficult. The majority of the Houston Ship Channel reefs which exist in 3 to 7 m of water were not surveyed for this reason; (3) Many of the leased areas were not surveyed. The circa-1970 survey concentrated on the known major reefs in the bay because of concerns at that time about shell dredging; (4) Upper East Bay and West Bay were not included in the survey. For West Bay, the existence of extensive areas of unconsolidated shelly mud was known to the survey team, but its areal extent made survey impractical with the standard poling method.

Two additional problems relate to the method used to define reef area. Our survey often identified small satellite patch reefs which were combined with the larger "parent" reef in our esti-

TABLE 4.

Estimated fraction of the total reef and unconsolidated shelly sediment in the Galveston Bay system contributed by anthropogenic activities.

Total Man-Made Reef				
	Area (in hectares)	Area (in sq. miles)	Percent of Total Reef	Percent of Total Man-made Reef
Total Man-made	1961.657	7.3	18.2%	—
Houston Ship Channel <sup>1</sup>	1092.230	4.2	10.1%	55.7%
Other Dredged Channels <sup>4</sup>	382.110	1.5	3.5%	19.5%
Oil Fields and Pipe Lines <sup>2</sup>	375.563	1.5	3.5%	19.1%
Artificial Reefs <sup>3</sup>	175.553	0.7	1.6%	8.9%

<sup>1</sup> Does not include extensive acreage adjacent to the channel forming part of South Redfish Reef.

<sup>2</sup> Rough estimate only. Little information exists to differentiate natural reef from reef originating from oil field development in these areas.

<sup>3</sup> Includes leases, reefs made for mitigation of shell dredging, reefs made to enhance the oyster fishery, etc. Rough estimate only; not all artificial reefs could be discretely identified, particularly in the Redfish Bar area, and little information exists to differentiate natural reef from man-made reef on leases.

<sup>4</sup> Channel-associated reefs in Bastrop Bay not included.

mates of reef area. Many such satellite reefs were not surveyed in the circa-1970 survey. Accordingly, best comparisons were made between reefs where most or all of the reef area was represented solely by the larger reefs surveyed in both instances. Second, some discrete reefs surveyed in circa 1970 had coalesced to form larger bodies. Under these conditions, only an approximate comparison could be made. In certain cases, Shuttle Reefs and Ernest Reef for example, the reef itself could no longer be identified even approximately and the name was deleted from the survey map. Such instances are not the result of reef loss, but of reef accretion and the improved precision of our surveying method.

Bearing these differences in mind, we can proceed to compare the results of the present survey with the circa-1970 survey of Benefield and Hofstetter (1976).

#### East Bay

Of the reefs that could be compared, the circa-1970 survey recorded 1111 hectares (Table 5). Our survey recorded 1215 hectares, an 8% increase in 20 yr. The uppermost reefs in the bay, Frenchy's Reef and Bob's Knob, lost a small amount of area; the remaining reefs were slightly larger. Overall, few reefs varied substantially in size. The 2 barrier reef tracts in East Bay, Middle/Gale's/Drum Village and Bull Hill/Hanna Reef, gained slightly. The large gain recorded for Pepper Grove Reefs was due to patch reefs that were not surveyed in the circa-1970 survey. Most of the small perpendicular reefs along the north shore were slightly larger in 1991. As both surveys were intensive in this area, this difference can be accounted for either by reef accretion or a slight variation in the definition of reef boundary between the 2 methods.

The East Bay area contained 2 uncharted reefal areas, a relatively large extension of Hanna Reef to the southeast towards Sievers Cove, not charted in the circa-1970 survey, and the upper bay patch reefs which were not surveyed at that time. Referral to charts and local accounts suggests that the patch reefs of upper East Bay, Tong Reefs for instance, have lost some acreage over the years as have Frenchy's Reef and Bob's Knob; however, no quantitative data are available.

Few data are available for comparison of relief. Reference was made to old charts where possible (U.S. Coast and Geodetic Survey 1855, 1907, 1921, 1924, 1957, National Oceanic and Atmospheric Admin. 1990). In general, the Hanna Reef tract has gradually deepened since 1850 with the majority of the deepening since the 1920s. The loss of shell banks, islands and shell bars is not unusual over this time frame (Marshall 1954) and may be explained, in this case, by regional subsidence (Gabrysch 1984, Jorgensen 1975, Ratzlaff 1982). The detachment of most reefs from the shoreline, a relatively unusual feature typical of most Galveston Bay reefs, can be explained by shoreline retreat that has accompanied subsidence in the area (Paine and Morton 1986, Morton et al. 1983).

However, depth and relief should not be confused. Perusal of old charts reveals that the relief of Hanna Reef has varied relatively little since 1850. Like most barrier reefs, the upestuary side has lower relief than the downestuary side. Old charts compare well with current observations that relief rarely exceeded 0.3 m on the upestuary side and was about 1.5 to 1.75 m on the downestuary side.

Benefield and Hofstetter (1976) and Benefield (1976) reported that parts of the Middle/Gale's/Drum Village barrier reef and its extension Pepper Grove Reefs, were heavily silted after shell

TABLE 5.  
Comparison of the 1991 and circa-1970 survey of East Bay  
(comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
East Bay			
Bob's Knob	3.359	2.030	0.60
Bull Hill <sup>2</sup>	76.568	114.052	
Buckshot Reefs <sup>1</sup>	4.614	8.852	1.92
Catfish Reef <sup>1</sup>	7.042	9.935	1.41
Cowshed Reef <sup>1</sup>	12.991	14.758	1.14
Drum Village Reef	47.916	37.508	
Frenchy's Reef <sup>1</sup>	83.246	74.879	0.90
Gale's Reef	63.901	95.578	
Hanna Reef <sup>1</sup>	526.103	475.067	
Lone Tree Reef <sup>1</sup>	7.446	8.288	1.11
Middle Reef	102.104	110.280	
Moody Reef <sup>1</sup>	13.760	15.900	1.16
North Bull Hill Reef <sup>2</sup>	20.801	59.160	
Pepper Grove Reefs	49.777	70.355	1.41
Richard's Reef <sup>1</sup>	12.262	17.833	1.45
Stephenson Reef <sup>1</sup>	12.262	10.545	0.86
Terry's Ridge <sup>1</sup>	13.395	17.268	1.29
Whitehead Reef <sup>1</sup>	18.575	32.399	1.74
Combined Reefs <sup>3</sup>			
Drum Village/Gale's/ Middle Reef <sup>1</sup>	213.921	243.366	1.14
Bull Hill/Hanna Reef <sup>1,2</sup>	623.472	648.279	1.04
Total <sup>4</sup>	1076.122	1174.687	1.09

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Parts of Bull Hill and North Bull Hill Reef included here were not included in estimate for East Bay in Table 3.

<sup>3</sup> Comparison of circa-1970 and 1991 boundaries uncertain for individual reef estimates. The combined estimate is more likely to be accurate.

<sup>4</sup> Does not include reefs unsurveyed in circa-1970. See Table 3 for full area estimates.

dredging just prior to the circa 1970 survey, which might explain the previously unsurveyed reef in the Pepper Grove area. Shell dredging removed a considerable fraction of the total reefal coverage in this area during the 1950s and 1960s (Rehkemper 1969, Quast et al. 1988). Although much of this area continues to have very low relief and dredge hauls often contain muddy shell indicative of continued silting in the area, our slightly larger areal estimates indicate that the reef tract has remained viable. The slightly larger areas for this barrier reef may accrue from the removal of silt since the circa-1970 survey or real accretion. In addition, one cannot exclude the possible value of the many oyster leases in the area in maintaining the viability of this reef tract. However, examination of old charts reveals that only low-relief reefs existed in this area throughout recorded time, so that the present low relief has been a persistent feature of this area regardless of the activities of man.

## Trinity Bay

Overall, Trinity Bay contained about 290 hectares more reef than surveyed in the circa-1970 survey (Table 6). As this area was replete with patch reefs and smaller satellite reefs near the larger reefs, most of which were not surveyed previously, only a few reefs offered direct comparisons. These fell into 3 categories: (1) some reefs changed little in areal extent, like Big Beezley Reef, Clamshell Reef and Dow Reef; (2) some had lost area, like Trinity Reef and Little Bird Reef, however the total area lost was small; (3) some had gained considerably, like Tidewater Reef, Outer Beezley Reef and Vingt-et-un Reef which about doubled in size and Lost Reef which was half again as large as in circa 1970.

Trinity Bay contains a number of artificial reefs most originating as mitigation for shell dredging (Benefield and Hofstetter 1976, Benefield 1976). Of these, all but Trinity Reef had gained some area over the last 20 yr. None had gained substantial area.

Several significant discrepancies existed between the 1991 and circa-1970 areal estimates. A large alongshore reef, referred to as Fisher and Elliotts Reefs in Figures 4 and 6, was probably incompletely surveyed in the circa-1970 survey. Our areal estimate is considerably larger. The large field of small patch reefs associated with the oil field around Old Yellow Reef was combined with this reef in our areal estimates, thus substantially increasing its esti-

TABLE 6.

Comparison of the 1991 and circa-1970 survey of Trinity Bay  
(Comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
Trinity Bay			
Big Beezley Reef <sup>1</sup>	30.109	30.927	1.03
Clamshell Reef <sup>1,2</sup>	4.087	4.330	1.06
Dow Reef <sup>1</sup>	32.335	40.830	1.26
Dryhole Reef <sup>1,2</sup>	6.313	5.746	0.91
Fisher Reef	69.122	123.757	1.79
Little Bird Reef <sup>2</sup>	1.619	0.349	0.22
Lonesome Reef <sup>1,2</sup>	7.204	8.222	1.14
Lost Reef <sup>1</sup>	6.677	10.831	1.62
Middle Beezley Reef <sup>1</sup>	9.672	12.075	1.25
Old Yellow Reefs <sup>2</sup>	2.590	19.976	7.71
Outer Beezley Reef <sup>1</sup>	9.308	20.674	2.22
Spoonbill Reef <sup>2</sup>	1.214	6.958	5.73
Tem Reef <sup>1,2</sup>	13.841	14.906	1.08
Tidewater Reef <sup>1</sup>	2.590	7.230	2.79
Trinity Reef <sup>1,2</sup>	7.446	6.072	0.82
Vingt-et-un Reef <sup>1</sup>	13.780	28.961	2.10
Total <sup>3</sup>	217.907	341.844	1.57

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Partially or fully originating from man's activities.

<sup>3</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

ated area compared to the circa-1970 survey. These patch reefs were not surveyed previously. Other patch reefs, including Ray's Reef, Little Beezley Reef, and Upper Beezley Reefs were also unsurveyed in circa 1970. In total, a discontinuous line of patch reefs covers much of the upper half of the mouth of the Trinity Bay, an area greater than 20 square miles and too large to be surveyed by the poling method used by Benefield and Hofstetter (1976). Finally, numerous small reefs reported, but not surveyed, in circa 1970 along the south Trinity shoreline were not found by our survey. In all likelihood, these reefs have disappeared over the last 20 yr.

The only comparison of relief afforded by the old navigational charts is Fisher Shoals, the relief of which is approximately the same as observed in 1855 (USCGS 1855). Evidence of subsidence comes from the shoreline detachment of most of the reefs and the likely origin of portions of Fisher and Elliotts Reef as former beach lines.

## Red Bluff/Morgan Point Embayment

Very few reefs in this area could be used for comparison between the 2 surveys. Of those that could be used, all showed slight to moderate growth in size over ~20 yr (Table 7). Larger discrepancies include the following: (1) Bayside Reef could not be relocated; (2) In all likelihood, reefs in the Cedar Bayou area were not adequately surveyed in the circa-1970 period as they exist as a discontinuous field of patch reefs at the mouth of the bayou and small reefs on the Cedar Bayou Channel spoil banks. In addition, some may be on new dredge spoil deposited since the circa-1970 survey; (3) No surveys were conducted in the East Red Bluff and Bayport Channel areas in circa 1970. Our survey found a significant number of patch reefs and reefs on spoil banks in this area.

TABLE 7.

Comparison of the 1991 and circa-1970 survey of the Red Bluff/Morgan Point Embayment (comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
Red Bluff/Morgan Point Embayment			
Bayside Reef	0.405	0.000	-
Bent Pipe Reef	7.811	12.859	1.65
Crow's Nest Reef	0.809	1.840	2.27
Red Bluff Reef <sup>1</sup>	28.248	29.979	1.06
Tin Can Reefs	5.221	10.095	1.93
West Red Bluff Reef <sup>1</sup>	4.087	5.859	1.43
Yacht Club Reef <sup>1</sup>	13.395	19.057	1.42
Total <sup>2</sup>	59.976	79.689	1.33

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

## Clear Lake Embayment

The total reef in this area has remained approximately constant since the circa-1970 survey; however, individual reefs changed dramatically in size (Table 8). Most reefs deep in the embayment lost significant reef area, including Bayview Reef, Courthouse Reef and Humble Reef. Some were noted to be silting up in 1970 (Benefield and Hofstetter 1976) so that subsidence and siltation are probably responsible for the lost reefal area. The Clear Lake Embayment has subsided more than most of the remaining parts of Galveston Bay (Jones and Larson 1975; Gabrysch 1984). Reefs farther out, like San Leon Reef, Halfway Reef and Smith Reef, gained area.

Both artificial and natural reefs gained acreage and both artificial and natural reefs lost acreage; hence, location rather than mode of origin was important. Most of the additional reefs included in the 1991 survey that were unsurveyed in circa 1970 were small patch reefs associated with oil field development and pipeline emplacement in the central part of the embayment. Once again, shoreline separation and the presence of alongshore reefs probably originated from regional subsidence and shoreline retreat.

## Dickinson Embayment

The amount of reef present in the Dickinson Embayment was significantly greater in our survey than in the circa-1970 survey for 4 reasons (Table 9): (1) Significant reef accretion occurred on a few reefs; (2) Several reefs, like Dollar Reef and April Fools Reef, were not completely surveyed in circa 1970. Additionally, both include substantial areas of semi-consolidated shelly sediment

TABLE 8.

Comparison of the 1991 and circa-1970 survey of the Clear Lake Embayment (comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
Clear Lake Embayment			
Bayview Reef <sup>1,2</sup>	12.626	6.302	0.50
Clear Lake Channel Reefs <sup>2</sup>	4.209	8.237	1.96
Courthouse Reef <sup>2</sup>	3.359	1.953	0.58
Eagle Point Reef <sup>1,2</sup>	17.078	14.725	0.86
Halfway Reef <sup>2</sup>	2.954	10.778	3.65
Humble Reef <sup>1</sup>	2.590	0.606	0.23
Little Scott Reef <sup>1</sup>	2.954	3.130	1.06
Pine Gully Reef	1.497	0.000	-
San Leon Reef <sup>1</sup>	24.889	34.385	1.38
Scott Reef <sup>1</sup>	14.488	13.478	0.93
Smith Reef <sup>1</sup>	3.723	9.732	2.61
Total <sup>3</sup>	90.367	103.326	1.14

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Partially or fully originating from man's activities.

<sup>3</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

TABLE 9.

Comparison of the 1991 and circa-1970 survey of the Dickinson Embayment (comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
Dickinson Embayment			
April Fools Reef	26.750	43.214	1.62
Crescent Reef <sup>2</sup>	8.175	3.621	0.44
Dickinson Channel Reefs <sup>2</sup>	2.550	40.330	15.82
Dickinson Reef <sup>1</sup>	78.026	64.028	0.82
Dollar Reef <sup>1</sup>	107.285	215.848	2.01
Experimental Reef <sup>2</sup>	2.954	2.344	0.79
Half Moon Reef <sup>1</sup>	14.852	16.717	1.13
Island Reef <sup>2</sup>	7.042	3.689	0.52
Levee Reef <sup>1</sup>	80.534	81.109	1.01
Little Half Moon Reef <sup>1</sup>	0.486	1.348	2.77
Marsh Reef	4.452	10.158	2.28
Moses Gate Reef <sup>2</sup>	1.052	6.262	5.95
Shoal Reef <sup>2</sup>	1.133	1.974	1.74
Todd's Dump and Redfish			
Island <sup>1,4</sup>	218.130	283.722	1.30
Total <sup>3</sup>	553.421	774.364	1.40

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Partially or fully originating from man's activities.

<sup>3</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

<sup>4</sup> Redfish Island included in the estimate of reef along the Houston Ship Channel in Table 3 rather than the estimate for the Dickinson Embayment. Redfish Island included here for comparison to estimates of Benefield and Hofstetter (1976).

which may not have been included in the earlier assessment; (3) The circa-1970 survey did not cover the central portion of the embayment and thus did not record reef associated with leases or the spoil banks along the Dickinson Channel; (4) Finally, 3 major reefs, Pelican Reef, Desperation Reef (termed Parallel Reef by Masch and Epsey 1967) and Resignation Reef, were not surveyed in circa 1970. Early navigational charts show some relief in those areas suggesting the presence of reef prior to the circa-1970 survey and Masch and Epsey (1967) record some reef in this area; however, as significant reef accretion occurred along the Houston Ship Channel nearby, the origin or significant enlargement of these reefs through growth since the circa-1970 survey cannot be ruled out.

With the exception of April Fools Reef, all nearshore reefs were detached from the shoreline as observed elsewhere in the bay, probably due to shoreline retreat. Rehkemper (1969) shows extensive reef south of Todd's Dump. No reef was recorded in this area by Benefield and Hofstetter (1976). We were also unable to identify reef in this region.

Dollar Reef occurs on all old navigational charts. Relief on Dollar Reef, about 1.7 m, has remained more or less constant since 1855 (USCGS 1855, 1907, 1921, 1924, 1957, NOAA 1990). Although Halfmoon Reef does not appear on the original

1855 navigational chart, it does so on all subsequent ones and relief has remained approximately the same as observed during our survey throughout that period of time.

#### West Bay/Pelican Island Embayment

These 2 sectors were not surveyed in the circa-1970 survey. The area contains 2 barrier reefs, Confederate/North and South Deer Island Reefs and Carancahua Reef. Several thousand hectares of shelly mud supported an important fishery in 1983–1984 and leases were located in both the Shell Island Reef and Deer Island Shell areas as well as on Carancahua Reef in and before the early 1960s. With the exception of North and South Deer Island Reefs and Confederate Reef, the reefs and shelly mud in West Bay and the Pelican Island Embayment are unproductive today. Accordingly, these large expanses of shelly mud were present prior to the circa-1970 survey, as were the 2 barrier reefs, Carancahua Reef and Confederate/North and South Deer Island. Carancahua Reef appears on the earliest bathymetric survey of the area.

Paine and Morton (1986) discuss the potential impact of the Texas City Dike in reducing circulation to West Bay, particularly restricting flow from Galveston Bay during periods of northerly and easterly winds. In all likelihood, this reduced flow has reduced oyster production in West Bay. Flow is an important requirement for oyster populations (Keck et al. 1973, Grizzle 1990, Powell et al. 1987). Burr (1929–30) also noted only limited production in the area in the 1920s. This too was after construction of the dike.

#### Satellite Bays of West Bay

Christmas Bay, Bastrop Bay, Oyster Lake, and Chocolate Bay were surveyed by pole. With the exception of the northeastern half of Christmas Bay, reef and unconsolidated shell were abundantly distributed throughout these smaller bays. Reefs included barrier reefs, alongshore reefs, and reefs perpendicular to the shoreline. Extensive reef buildup had also occurred along all dredged channels. Unlike most other areas of the Galveston Bay system, the substantial areas of unconsolidated shell were often composed of scattered oyster clumps partially buried in the muddy substratum. This bottom type is common in smaller bays along much of the Texas coast.

No previous surveys have been conducted in these bays, so that historic trends in reef area cannot be determined. Extensive reef development along channels in Bastrop Bay and Chocolate Bay, however, indicates that reef accretion has occurred in some areas of these bays during this century. With few exceptions, relief of the reefs was less than 0.5 m. Average bay depths rarely exceeded 1.5 m, however. Depths obtained from previous charts of the area suggest little change in relief, to the extent changes can be determined in these poorly surveyed bays.

#### North Redfish Bar

Extensive coverage of patch reefs and consolidated patch reefs exists north of Redfish Bar (Table 10). This area was not extensively surveyed in the circa-1970 survey so that the apparently large increase in reefal area cannot unequivocally be considered true reef accretion during that time. Rehkemper (1969) noted some reef in this area in his mid-1950s survey. However, some reefs present in the circa-1970 survey, including Shuttle Reefs and Ernest Reef, could not be distinguished today within an extensive area of coalesced patch reefs, suggesting that local consolidation of patch reefs has occurred since circa 1970. Moreover, certain

TABLE 10.

Comparison of the 1991 and circa-1970 survey of the North Redfish Bar sector (comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
North Redfish Bar			
Bar 24 Reefs	1.902	2.226	1.17
Bart's Pass <sup>1</sup>	95.508	68.956	–
Bart's Pass West <sup>2</sup>	7.811	81.325	–
Possum Pass <sup>1</sup>	33.428	72.181	2.13
Roberts Reef	3.318	10.884	3.28
Sheldon Reef <sup>1</sup>	20.801	46.164	2.22
Combined reefs <sup>4</sup>			
Bart's Pass/Bart's Pass West <sup>1</sup>	103.319	150.281	1.45
Total <sup>3</sup>	162.768	281.736	1.73

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Partially or fully originating from man's activities.

<sup>3</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

<sup>4</sup> Comparison of circa 1970 and 1991 boundaries uncertain for individual reef estimates. The combined estimate is more likely to be accurate.

large natural reefs, Sheldon Reef and Possum Pass Reef, have also increased considerably in size. Some of this increase, however, was contributed by less consolidated shelly sediments which may not have been included in the circa-1970 survey. No clear evidence of reef loss since circa 1970 exists in the area.

Of particular note is the relatively limited amount of reef along the Chambers County line, once the location of the original barrier reef in the bay, originally called Redfish Bar (USCGS 1855, 1907, Eckhardt 1969). Charts through 1927 show a barrier reef, Redfish Bar, extending from Eagle Point (Edwards Point on the old charts) to Smith Point (Figure 3). Three passes permitted water flow through this barrier reef complex; West Pass, Middle Pass, and Opossum Pass. Only West Pass, which still exists behind Redfish Island, was deeper than ~1.7 m. Stories of cattle drives across Redfish Bar can certainly be substantiated by the bathymetry of the time. That this barrier reef acted as a significant impediment to water flow and salt transport is substantiated by Burr's (1929–30) description of the steep salinity gradients across the bar.

The only present-day remnant of this original barrier reef is Todd's Dump running from Eagle Point to Redfish Island. East of the Houston Ship Channel, the present-day equivalent, still called Redfish Bar, is centered between 1 and 2 miles south of where this original bar is located. The Chambers County line where the original bar was located is noteworthy for having only a few scattered patch reefs along its extent from Redfish Island to Smith Point.

The original Redfish Bar is no longer present on the 1957 navigational chart (partially based on a circa-1940 bathymetric survey) (USCGS 1957), but is still present on the 1927 chart (based on late 1800s and 1920s bathymetric surveys) and is specifically described by Galtsoff (1931) in his late 1920s survey of

Texas oyster reefs, so that the bulk of the original barrier reef probably disappeared between 1935 and 1945. L. Benefield stated that the U.S. Military removed shell from Redfish Bar for base construction during World War II, but exact records are unavailable. Records of shell dredging are insufficient to determine whether shell dredging was responsible, but it is curious that the western most portion, Todd's Dump, and the other large Galveston Bay barrier reef tract, the Hanna Reef tract, have remained in approximately the same location and, with few exceptional spots, of about the same areal extent and relief as can be estimated from the original 1850s survey (after 1963, however, these reefs were protected from shell dredging by the Texas Parks and Wildlife Department's designation of them as "major reefs"). Although significant natural changes in reefs can occur over half-century time scales (Marshall 1954), it seems likely that shell removal must have been a contributing cause in the disappearance of the original barrier bar.

### Redfish Bar

Significant areas of accretion and loss were observed along the present-day Redfish Bar and the northerly extension of the Hanna Reef tract (Table 11). Both the natural and man-made reefs in the area offered examples of accretion and loss, once again demonstrating that location, not mode of origin, is of greatest importance in determining the change in acreage since circa 1970.

The principle area losing acreage since circa 1970 was the Mattie B. and Tom Tom Reef portion of the Hanna Reef tract. Old charts suggest that this area has been losing acreage continuously since early in the century. At one time, only 2 natural passes existed through the Hanna Reef tract, Ladies Pass and Moodys Pass (USCGS 1907). It is likely that a new natural pass has gradually been formed in the Mattie B./Tom Tom Reef area by the outflow of the Trinity River, as discussed later.

Reef accretion and patch reef coalescence has occurred throughout the remainder of the Redfish Bar area and the northern extent of the Hanna Reef tract, particularly concentrated along the southern margin. The circa-1970 survey was particularly intensive in the Redfish Bar area. As most of the accretion is enlargement rather than the finding of new reefs, it is likely that the bulk of the ~500 hectares of new reef observed has accreted in the last 20 yr.

Incipient reef accretion on the southern edge of Bull Hill was noted as a shelly crust over mud and shelly mud. A lobe of sediment extending south of this area was noted during surveying to have scattered shell on it, although not enough to qualify as unconsolidated shelly substrate. This scattering of shell may represent the beginnings of continued reef expansion south of Bull Hill. Most of the satellite reefs around South Redfish Reef, like Triangle Reef, Missing Reef, "C" Reef, and Archie's Reef, can no longer be easily distinguished from South Redfish Reef. Smaller reefs like Slim Jim Reef and Pasadena Reef have increased dramatically in size. South Redfish Reef has nearly doubled in size with most accretion occurring along the southern margin. New reef, the Lost Beezley Reef area, has accreted south of the primary barrier bar in a line with Pasadena Reef, continuing a near century-long southerly progradation of Redfish Bar. Rehkemper (1969) recorded no significant shell deposits between Pasadena Reef and Redfish Bar. Neither did Benefield and Hofstetter (1976). These low relief reefs north and east of Pasadena Reef indicate the beginnings of a major new reef complex in that area.

TABLE 11.  
Comparison of the 1991 and circa-1970 survey of Redfish Bar  
(comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
Redfish Bar			
Archie's Reef	3.723	3.426	0.92
Bull Shoals <sup>1</sup>	34.925	40.264	1.15
"C" Reef	3.723	5.432	1.46
Dee's Reef	2.954	8.174	2.77
East Redfish Reef <sup>1</sup>	31.607	82.014	2.59
Four Bit Reef <sup>1,2</sup>	9.672	9.206	0.95
Gaspape Reef <sup>1,2</sup>	14.124	20.687	1.46
Mary's Reef	8.174	18.414	2.25
Mattie B. Reef <sup>1</sup>	20.801	14.938	0.72
Missing Reef <sup>2</sup>	9.308	9.175	0.99
North Redfish Reef <sup>1</sup>	202.914	237.984	1.17
Pasadena Reef <sup>1</sup>	12.950	49.853	3.85
Santa Reefs	3.359	7.112	2.12
Slim Jim Reef <sup>1</sup>	8.903	31.236	3.51
South Redfish Reef <sup>1,4</sup>	371.874	702.109	1.89
Tom Tom Reef <sup>1</sup>	5.585	3.573	0.64
Triangle Reef <sup>2</sup>	3.723	7.796	2.09
Total <sup>3</sup>	748.319	1251.393	1.67

<sup>1</sup> Best comparison; other comparisons of limited usefulness because reef portions included in estimate might be different from, or because entire area included in 1991 estimate was not mapped by, Benefield and Hofstetter (1976).

<sup>2</sup> Partially or fully originating from man's activities.

<sup>3</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

<sup>4</sup> Portion of South Redfish Reef located on the Houston Ship Channel spoil banks included in this estimate, rather than in Table 12.

The rate of reef accretion is likely dependent on the subsurface geology in the area, some portions of which include >10 m of soft mud (Beneficial Uses Group 1992). The higher rate of reef accretion on the southwestern section of Redfish Bar, as compared to the Bull Hill area, is probably due to the lower stability of the soft bottom south of Bull Hill.

Long-term changes in relief are more evident in the Redfish Bar sector than elsewhere. North of the present-day Redfish Bar, along the Chambers County line, considerable loss of relief has occurred, on the order of 1 to 2 m depending on location and bathymetric survey. On the present Redfish Bar, relief has increased, although exact quantification is difficult. Pasadena Reef has existed since at least 1855 (USCGS 1855), at that time called Hannah Island. Hannah Island disappeared prior to 1921 (USCGS 1907, 1921) by which time up to 1 m of relief had been lost in the area. Regional subsidence was probably not responsible for this change since the bulk of the Redfish Bar and Hanna Reef tract shell islands were still present at that time. No further changes in relief on Pasadena Reef can be clearly differentiated since that time. Significant reef accretion on the northeastern side of Pasadena Reef and the formation of Lost Beezley Reef has not yet resulted in a significant increase in relief of this area. Fathometer

traces, in fact, demonstrate a flat bottom over most of this area despite its oyster substrate.

### Houston Ship Channel

Over 1000 hectares of reef were identified along the Houston Ship Channel (Table 12). From about Buoy 63 to Morgan Point, the majority of this reef exists between the channel edge and the crest of the spoil banks paralleling each side, between the 2-to-3 m and 5-to-7 m depth range. Little reef coverage exists on the outer slope of the spoil banks in this reach. We made no effort to survey above Morgan Point; reef certainly exists in this area (e.g., site GBSC in Wilson et al. 1992). From Buoy 63 to approximately Buoy 47, reef extends out from the ship channel edge across the parallel-trending spoil banks and grades into the Redfish Bar reef tract and the reef in the Dickinson Embayment. The extension of reef across the spoil banks occurs incrementally from Buoy 63 south as reef gradually extends farther and farther down the far or bay side of the spoil bank from its crest to the surrounding natural bottom. Establishing boundaries for computing reef acreage in these areas proved difficult, so estimates of along-channel reef must be considered conservative in this reach.

Comparison to the circa-1970 survey shows a substantial increase in reef coverage along the entire channel from Buoy 47 to Morgan Point. Difficulties in surveying by pole limited the circa-1970 survey and this limitation may account for a considerable portion of the inequity. However, substantial accretion near the crests of the spoil banks from Buoy 63 to about Buoy 47 certainly would have been observed circa 1970 so that the evidence suggests dramatic reef growth over the last 20 yr along the ship channel.

The Houston Ship Channel has been enlarged many times since its creation around the turn of the century (USCGS 1907, 1921, 1924, 1957, NOAA 1990). The last significant enlargement occurred in the early 1960s. Although one cannot be sure of the effects of that enlargement on the entire spoil bank system, a reasonable conclusion is that the majority of the >1000 hectares of

reef along the Houston Ship Channel has accreted over the last ~30 yr.

## DISCUSSION

### Circa-1970/1991 Survey Comparison

Comparison of the present 1991 survey with the circa-1970 survey of Benefield and Hofstetter (1976) reveals the following trends.

(1) Our survey approximately doubles the known area of reef and unconsolidated shelly substrate in the bay system. A substantial fraction of this newly surveyed reef and unconsolidated shelly sediment was present but not surveyed in circa 1970. However, among those reefs where a precise comparison was possible between the 1991 and circa-1970 surveys, reef accretion rather than reef loss was the general rule. Reef accretion was most noticeable in 3 areas; along the Houston Ship Channel, at the southern edge of Redfish Bar and the Bull Hill extension of the Hanna Reef tract, and in the Dickinson Embayment.

(2) Reef loss, although minor overall, was concentrated in 4 areas; along the southern shore of Trinity Bay, in upper East Bay, in the Mattie B./Tom Tom Reef area at the northern end of the Hanna Reef tract, and in the inner portion of the Clear Lake Embayment.

(3) Reefs originating through man's activities, whether associated with spoil banks of channels, oil field development, or purposefully created (artificial reefs), did not vary any differently than natural reefs. Rates of accretion and loss were location specific rather than dependent on the mode of origin of the reef. Clearly, artificial reefs can be markedly successful, if sited correctly to enhance reef growth.

(4) The data available to assess changes in relief are poor. Nevertheless, the comparisons that can be made indicate substantial changes in relief in only one area, Redfish Bar, which has, for all intents, prograded south since the turn of the century. Relief on the remaining barrier reefs has not changed perceptibly. Depth, of course, has, but depth changes are mostly related to regional subsidence. The single possible exception is in the vicinity of Mattie B. Reef. Saying this does not necessarily discount the overall impact of shell dredging prior to circa 1970; however, most of that effort was not concentrated on the barrier reefs (Benefield 1976; Masch and Espey 1967) which were usually the only reefs indicated on navigational charts. The causative reason for the disappearance of the original Redfish Bar cannot be precisely identified nor are data sufficient to document the possible recovery of the many smaller reefs in East Bay and Trinity Bay that were impacted by shell dredging prior to 1970.

(5) Most vessels in the oyster fishery of Galveston Bay use heavy 1 to 1.3-m toothed dredges towed slightly forward of amidships while running in a tight, circular arc. The oyster fishery might impact relief and areal extent; relief because shell is removed, areal extent because shell might be redistributed off reef onto leases or the sides of fished reefs. Once again, appropriate data for comparison are meager. No shell budget is available for any reef [shell budgets are reviewed in Powell et al. (1989) and Cummins et al. (1986)] so that the fraction of shell produced that is removed by the fishery, the fraction destroyed naturally by taphonomic processes, which is likely to be substantial, and the fraction preserved and thus available as cultch in subsequent years is unknown. However, effects as large as Marshall's (1954) esti-

TABLE 12.

Comparison of the 1991 and circa-1970 survey of the Houston Ship Channel sector (comparisons computed as 1991 area/circa-1970 area).

Comparison of 1970 and 1991			
Reef	Circa 1970 Area (From Benefield and Hofstetter 1976)	1991 Area	Fractional Change
Houston Ship Channel <sup>1</sup>			
Morgan Point Reefs <sup>2</sup>	0.405	14.122	34.87
"53" Reefs <sup>2</sup>	15.945	177.977	11.16
"59" Reefs <sup>2</sup>	56.495	192.746	3.41
"63" Reefs <sup>2</sup>	26.022	146.898	5.65
Total <sup>1</sup>	98.867	531.743	5.38

<sup>1</sup> Does not include reefs unsurveyed in circa 1970. See Table 3 for full area estimates.

<sup>2</sup> Partially or fully originating from man's activities.

<sup>3</sup> Reef adjacent to Redfish Island and that portion of South Redfish reef on the spoil bank not included in these estimates. See Table 3 for full area estimates.

mates from Virginia of a relief reduction of  $0.17 \text{ m yr}^{-1}$  due to the fishery would have been observed in a comparison of our 1991 survey to earlier bathymetric surveys.

No evidence exists to support concerns that shell removal by the fishery is an important process in reducing relief or areal extent of oyster reefs (Quast et al. 1988). Supporting evidence from the 1991 survey includes the following: (1) some of the most heavily fished reefs have clearly not varied much in relief since the original 1850s survey (USCGS 1855); (2) Relief of open and closed (by coliform counts) reefs (TDH 1992) does not vary uniformly. Some of each have relatively high relief (1–1.5 m) and low relief ( $<0.5 \text{ m}$ ). Relief is primarily controlled by local conditions and individual reef history; (3) On the average, heavily fished reefs have accreted more area in the past 20 yr than reefs not fished; (4) The most significant areas of reef loss are in closed areas of the bay (TDH 1992).

The data do demonstrate several likely impacts on reef area by the fishery. Most leases today contain reef or semi-consolidated shelly areas. At least some of this material originates from shell transplanting by the fishery. As these areas have accreted or lost as much as natural reef, their survival is, once again, dependent on siting, not mode of origin. Movement of shell off reef edges, if anything, has aided in reef growth. Many reefs are accreting area at their margins. Some unknown portion of this accretion may be due to shell movement by the fishery. We see no evidence of reef loss by this mechanism.

Accordingly, over all, areal extent of reefs has probably been increased by fishing activities. The evidence suggests that judicious siting of new leases and requirements for private shell planting on leases could substantially increase the acreage of reef in the Galveston Bay system.

(6) Relief varies considerably between the 2 sides of most reefs so that reference must be made to the area of the surrounding bay used to define relief. We have routinely used the surrounding bay bottom on the downestuary side. The upestuary side typically has lower or no relief. The downestuary side frequently, but not always, has substantial relief. The reason for the difference is probably the damming of sediments behind these reefs on the upestuary side. Although this mechanism certainly should result in the loss of reef, many reefs which have had little or no relief on the upestuary side for many years varied little in areal extent between 1991 and circa 1970 or even grew slightly. The positive role of leases in maintaining reefs above the surrounding bay bottom in these areas should also be considered.

#### *Persistence, Malleability and Modifying Agents*

Certain components of the Galveston Bay reef system have persisted throughout recorded time; others have exhibited substantial malleability, changing position and shape over time spans of a half century or so in response to natural and man-made changes in the bay system. Besides the difficulty of assessing changes produced by shell dredging and the likely local impacts of shell transfer to leases and artificial reefs, 2 regional impacts seem preeminent.

(1) Regional subsidence has resulted in the increase in depth and areal extent of the Galveston Bay system. The products of regional subsidence are threefold: (a) Most reefs are detached from the shoreline, a likely result of subsidence and shoreline retreat; (b) Regional subsidence has increased the depths over the reefs thus (i) reducing the acreage intertidally and subacrially exposed

particularly on the barrier reef tracts and (ii) drowning alongshore beach deposits that have later developed into alongshore reefs. Arguably, increased depth has increased bay productivity by increasing subtidal acreage and increasing water velocity over the majority of the barrier reefs. A comparison of productivity between Galveston Bay reefs and those typical of the lower bays, many of which are currently intertidal (Copeland and Hoese 1966), would be instructive; (c) Areas of high subsidence, such as the Clear Lake Embayment, have suffered reef attrition due to siltation. However, of necessity, this area must also no longer be adequate to support reef growth; otherwise, reef growth should have kept up with siltation. What besides subsidence has reduced the area's viability is unclear.

(2) Channelization, dike construction, and loss of the original Redfish Bar have substantially changed bay circulation pattern. The Texas City dike has probably reduced circulation in West Bay. The pre-1900 circulation pattern in Galveston and Trinity Bays is unknown. Certainly today's circulation must differ substantially from that time if for no other reason than that the original barrier reef dam, Redfish Bar, no longer exists and the Houston Ship Channel has been added. Redfish Bar, as it existed pre-1900, had 3 primary channels, only one of which, West Pass, was probably deep enough to permit substantial water flow upestuary and downestuary. A significant salinity gradient existed over this bar (Burr 1929–30, Galtsoff 1931). In addition, the Houston Ship Channel has modified the flow structure and isohalines in the bay which now run more or less parallel to the ship channel rather than across bay as they likely did originally over much of the bay's extent.

As a consequence, today, the bulk of the Trinity River flow, the primary freshwater source to the bay, exits Trinity Bay along the southern shore, wraps immediately around Smith Point, and flows downestuary across Mattie B. Reef and Tom Tom Reef, reaching nearly to Bolivar Peninsula before becoming entrained in the outward flowing water at the inlet (Fig. 1). This circulation pattern has likely existed for many decades (Reid 1955, Diener 1975) although its intensity must have increased as the Houston Ship Channel was deepened and the Redfish Bar dam disappeared. The result of this changing flow pattern has been (a) loss of a number of small reefs along the southern shore of Trinity Bay, (b) the demise of the Hanna Reef tract in the vicinity of Mattie B. Reef and Tom Tom Reef, the present outlet for much of the Trinity River flow, and (c) the accretion of reef along the southern edge of South Redfish Reef, the western and northern trend of Pasadena Reef, and the southern edge of Bull Hill and associated reefs. These latter 3 areas adjoin the present route of outflow of the Trinity River as it crosses the present barrier reef complex in the bay and, thus, follow the new isohaline structure of the bay.

Each of these changes is a response to changing water flow and salinity that has shifted the bay's geology (the reefs) out of equilibrium with the bay's flow structure. Some reefs are no longer optimally located for continued high productivity; many areas of low reef coverage would now support productive reef if substrate became available. One can expect a continued response to the changed flow and salinity regime in these areas in decades to come as the bay continues to develop a new equilibrium condition. However, our observations suggest that reef builds slowly out onto muddy bottom. The rates of taphonomic processes can be expected to be high in these areas (Powell et al. 1989) so that the natural process of reef accretion may be slow. Moreover, these outgrowing margins, especially south of South Redfish Reef, may not

withstand significant dredging by the fishery depending upon the extent of substrate consolidation, which is not currently known. Careful management of these areas would be prudent.

The Houston Ship Channel has extended the isohalines up-estuary to the great benefit of oyster populations and the oyster fishery. In effect, the Houston Ship Channel has extended the area of oyster productivity much beyond that which would have existed prior to channelization. Like the removal of the pre-1900 Redfish Bar, which probably restricted the areal extent of the key 15‰ isohaline (U.S. Army Corps of Engineers 1987), the Houston Ship Channel has expanded and modified the isohaline structure and increased water velocity, both conducive to oyster growth. Over 1000 hectares of reef have developed along this channel, a substantial fraction of which extends between the channel edge and the crest of the parallel-trending spoil banks. In the reach from Buoy 63 to Morgan Point, all reef development is in this zone.

The data show the overriding importance of the coincidence of 2 bathymetric features for development of reef along channels. A channel is, of course, required. However, a spoil bank is also required. Observation of channels in which spoil was placed on only one side always shows that reef development is predominantly or exclusively on that side.

Interestingly, the expected increase in predation and disease with increased salinity (Ray 1987) is not necessarily an overriding influence on reef survival. A healthy oyster population with adequate food and adequate water flow can outgrow predators and diseases. High salinity reefs like Confederate Reef offer readily observable proof of this fact. One interesting oddity of the Galveston Bay system is the presence of most reef accretion down-estuary of the 15‰ isohaline suggesting that salinity and its associated factors such as oyster disease and predation, though important, are not the overriding determinants controlling oyster population expansion in Galveston Bay. Food supply is an overriding influence on reef productivity in this system (Powell et al., in press).

Finally, the Houston Ship Channel has created a barrier separating the Trinity River-affected eastern part of Galveston Bay from the western part of Galveston Bay. This "dam," affects the distribution of food, turbidity and current flow. In particular, on the average, the western part of the bay is less turbid and the differential is significant in that it is in the range of values that substantially affect filtration rate in oyster populations (our unpubl. data). The substantial accretion of reef in the Dickinson Embayment is almost certainly a result of the last remnant of the original Redfish Bar (Todd's Dump) and the Houston Ship Channel isolating this area from the generally higher turbidity elsewhere in the bay.

### CONCLUSIONS

Overall, Galveston Bay has accreted substantial reef in the last 20 yr. The location and mechanisms of reef accretion suggest that natural responses to changes in circulation and salinity by the oyster populations are primarily responsible rather than the direct production of new reef by man. These responses have been primarily induced, however, by both natural and man-made events. These include (a) the construction of the Houston Ship Channel and the Texas City Dike, (b) removal, by mechanisms not well documented, of the original Redfish Bar, (c) regional subsidence deepening the bay and facilitating shoreline retreat, and (d) the placement of spoil along dredged channels providing suitable sub-

strate for settlement. Local effects like leases, artificial reefs and, in many areas, shell dredging, have had less impact over the last 20 yr, although shell dredging significantly modified reef coverage in some areas prior to 1970.

Whether bay productivity has increased commensurately with the increased acreage cannot be assessed without recourse to a population-dynamics model. Some reef has formed in presently optimal locations, yet other reef, still extant, finds itself in areas of reduced quality. With the exception of the southern shore of Trinity Bay, the Clear Lake Embayment, the Mattie B./Tom Tom Reef area of the Hanna Reef tract and upper East Bay, conditions are not so poor as to result in loss of acreage. However it is not at all clear how much productivity is required to balance the natural and anthropogenically mediated taphonomic processes that continually destroy shell carbonate. Accordingly, the significant reef accretion documented by this survey should not be construed as clear evidence for increased productivity in Galveston Bay as a whole. Although productivity has dramatically increased in certain areas of the bay, productivity may have decreased commensurately in other areas of the bay.

Over the long term, however, circumstantial evidence indicates a significant increase in oyster production since the 1940s. Galtsoff (1931) remarks that Galveston Bay was not generally productive for the oyster fishery prior to 1930. Most Texas oysters came from more southern bays, i.e., Matagorda Bay and San Antonio Bay. Low productivity, according to Galtsoff (1931), resulted from the presence of a large barrier reef dam, Redfish Bar, that restricted bay circulation and compressed the bay's salinity gradient. The present Galveston Bay oyster fishery began in earnest in the mid-1950s. It is, perhaps, no coincidence that this increase followed the removal of the barrier dam and benefited further from the widening and deepening of the Houston Ship Channel, which together dramatically broadened the bay's salinity gradient. Accordingly, the present productivity of Galveston Bay is almost certainly a result of human intervention, albeit accidental, which has fortuitously improved the bay's circulation pattern by expanding the salinity gradient and increasing water flow through the central portion of the bay.

The apparent geological stability of reef distribution in a bay like Galveston Bay is a misinterpretation brought about by the observation of large masses of seemingly stable carbonate formed by oysters in the bay. In reality, over decades to half-century time scales, oysters are capable of substantially realigning oyster reef tracts in response to a changing environment. Under these conditions, which exist in Galveston Bay today, the presence of geologically significant oyster reefs should not be equated with productivity or with optimal living conditions for oysters. Such an equation is only defensible when the geological distribution of reefs is in equilibrium with the bay's hydrodynamics, which is certainly not the case today in Galveston Bay.

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## POPULATION AND DISEASE DYNAMICS OF MARYLAND OYSTER BARS: A MULTIVARIATE CLASSIFICATION ANALYSIS

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**ABSTRACT** Cluster analysis was applied to size frequency, spatfall, mortality, and disease prevalence data collected from 64 oyster bars in Maryland's Chesapeake Bay during 1990–1993. Six population types were identified. Salinity and prevalence of *Perkinsus marinus* and *Haplosporidium nelsoni* infections were important variables explaining cluster membership (stepwise discriminant analysis). Six similar population types were identified by cluster analysis of the same data set with *P. marinus* and *H. nelsoni* prevalence, mortality, spatfall, relative abundance of small and market oysters, and mean shell height as the population attributes. Both analyses pointed to recruitment, along with mortality from parasitic infections, as defining oyster population structure. Mapping cluster membership of each oyster bar over the 4 years provided a simple way to visualize year to year changes in many variables describing the oyster population.

**KEY WORDS:** *Crassostrea virginica*, population structure, *Perkinsus marinus*, *Haplosporidium nelsoni*, Chesapeake Bay

### INTRODUCTION

Historically, oyster populations in Maryland's Chesapeake Bay were controlled primarily by recruitment, which varied greatly from year to year (Meritt 1977), and harvest pressure, although predators and environmental extremes undoubtedly exerted some influence. Annual landings varied, but in the long term they were reasonably stable for decades after the exploitive harvests of the late 19th and early 20th centuries (Kennedy and Breisch 1981). In the past two decades, however, mortalities associated with the 2 protozoan parasites *Perkinsus marinus* and *Haplosporidium nelsoni* have become major influences on the abundance and size structure of oyster populations, with a concomitant decrease in landings to historic lows (Andrews 1988, Ford and Haskin 1988, Krantz 1993, Smith and Jordan 1993).

With the growing influence of the parasites, generalizations about the structure of oyster populations became more difficult, whether on a spatial or temporal basis. Four "management zones" were defined by Krantz (1992, 1993), largely on the basis of recruitment history and potential risks from *P. marinus* and *H. nelsoni* infections, factors which in turn were closely related to salinity patterns. In 1990, Maryland instituted modifications to its annual oyster monitoring program so that consistent data on oyster populations and parasitic infections would be obtained every year (Smith and Jordan 1993). The availability of consistent data over 4 years presented an opportunity for statistical approaches to identification of spatial and temporal patterns in the oyster populations. This report describes a group of multivariate analyses designed to investigate patterns of size structure, mortality, recruitment and parasitic infections among 64 oyster bars for a period of 4 years (1990–1993). The guiding idea was that patterns, or classifications, defined by biological variables would provide more information than those determined purely by geographical regions or salinity ranges. The resulting classifications could be useful in managing the oyster resource in Maryland.

### METHODS

#### Survey Sites and Methodology

An annual fall survey of Maryland Chesapeake Bay oyster populations has been conducted since 1939 (Krantz 1993). The

traditional survey has employed a single oyster dredge sample from each of many oyster bars, with sites chosen each year according to management interests. In 1990, the survey was modified so that a standard subset of 64 oyster bars (Fig. 1) was sampled more intensively (Smith and Jordan 1993). Annual mortality, as estimated by counts of "boxes" (empty oyster shells with both valves), spat densities, and counts of live oysters per unit volume of bottom material, along with depth, temperature, and salinity, were recorded at each site. Shell heights were measured to the nearest 5 mm for all oysters collected at the 64 sites. Spat were counted, but not measured in 1990 and 1991, so measured size distributions of spat for 1992 and 1993 were used in combination with actual spat counts for 1990 and 1991 to estimate (by proportions) spat size distributions for the earlier years. In 1990 and 1991, summer samples from a few sites were included in the data in addition to the standard fall samples.

Prevalences of parasitic infections were estimated from samples of 30 oysters from each of a subset of 43 of the 64 intensively sampled sites (Fig. 1). The majority of *P. marinus* diagnoses were done by incubating hemolymph samples in Ray's fluid thioglycolate medium (RFTM); the majority of *H. nelsoni* diagnoses employed hemolymph samples stained with hematoxylin and eosin. Some of the samples from 1990 and 1991 were diagnosed for *P. marinus* by incubation of rectal tissue in RFTM, and for *H. nelsoni* by fixed tissue histology. For analytical purposes, the diagnostic methods were combined so that prevalence estimates were available for all sites and years. Where more than 1 method was employed for a given sample, preference was given to hemolymph diagnosis. Although rectal diagnosis for *P. marinus* has been thought to be somewhat more sensitive than the hemolymph method, 2 recent studies have concluded that the methods yield equivalent prevalence estimates (Bushek et al. 1994, Calvo et al., submitted).

#### Statistical Methodology

Oyster size classes ranged in increments of 5 mm from 2–167 mm in shell height (a few of the larger size classes had zero counts for all sites and years; these were excluded from analysis). Each oyster also was classified as live, box, or spat by the biologists on

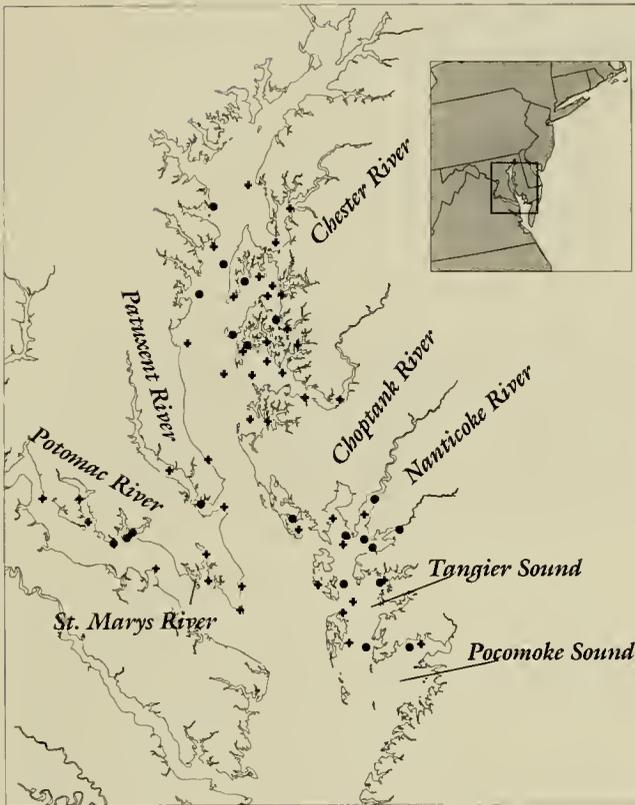


Figure 1. Location of oyster bars monitored in the Maryland Modified Fall Oyster Survey. Sites indicated by crosses were monitored for diseases and population attributes; sites indicated by circles were monitored only for population attributes.

board the sampling vessel. There was some overlap in the smaller size classes between oysters classified as spat and those classified as "smalls" (older than one year but <77 mm). Dead spat (spat boxes) were recorded, but observations were so sparse that this group was excluded from analysis.

The SAS procedure FASTCLUS (SAS Institute, Inc. 1988) was used for cluster analysis. This procedure allows the analyst to specify the number of clusters to be computed; the procedure was used iteratively to determine both the optimum number of clusters and the seeding method that gave the most satisfactory results. The optimum number of clusters was pre-defined as the largest number that did not result in clusters with only one or a very few observations. The seeding method was selected based on the same criterion. The analyses presented here were seeded by pseudo-random selection of observations from the data set. The size-frequency cluster analysis used counts of oysters in each 5 mm size class and category (e.g., 2–6 mm spat, 72–76 mm live oysters, 102–106 mm boxes) as attributes to be clustered; there were 67 of these variables. Because the variables were all similar (counts) they were not transformed or standardized for the size frequency analysis. Although this decision may have introduced some statistical bias because of unequal variances, the results could be portrayed as ordinary size-frequency histograms for ease of interpretation.

Stepwise discriminant analysis (SAS procedure STEPDISC; SAS Institute 1988) was used to determine if cluster membership from the size frequency cluster analysis could be explained by a set of autonomous variables. Temperature, depth, salinity, *H. nelsoni*

prevalence and *P. marinus* prevalence were used as potentially explanatory variables, with cluster numbers as classification variables. For the 21 of 64 sites where parasite prevalences were not monitored, prevalences were imputed as the cluster means from the population attributes cluster analysis (described below), so a variable representing the number of imputed values for each observation (0, 1, or 2) was included to determine if this procedure made a significant (spurious) contribution to cluster membership.

The population attributes cluster analysis used 7 variables: *H. nelsoni* prevalence, *P. marinus* prevalence, annual mortality (number of boxes divided by total number of oysters), number of small oysters per bushel of sample, number of market oysters ( $\geq 76$  mm) per bushel, number of spat per bushel, and average shell height. These variables were chosen from a much larger set contained in the monitoring database based on exploratory analysis and judgement. Disease prevalence and mortality data were transformed to arcsine( $\sqrt{p}$ ), where  $p$  was the proportion of the sample infected; spat densities were transformed to  $\log_{10}(y + 1)$ , where  $y$  was the raw count and 1 was added to remove zeros. All of the variables were then standardized to a mean of 0 and standard deviation of 1 before clustering.

## RESULTS

Baywide mean salinity, recorded at the time of sampling (October–November), varied somewhat over the 4 years (Fig. 2). Averaged by site over 4 years, salinity was highest in Tangier and Pocomoke Sounds, generally decreasing towards the northern Bay and in an upstream direction in the tributaries (Fig. 2).

### Size Frequency Analysis

The size frequency analysis produced 6 clusters of observations, based upon the criteria described above. Cluster means for

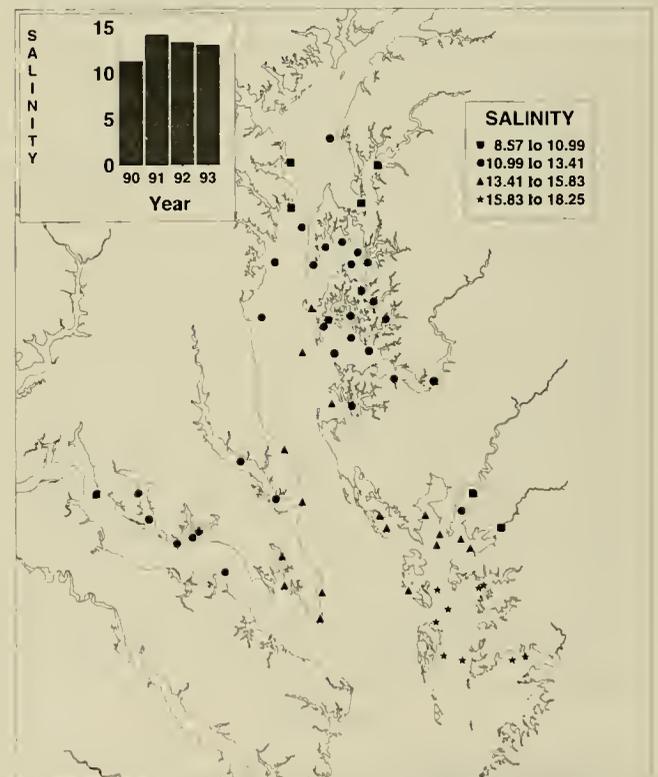


Figure 2. Ranges of mean salinity by site (4-year averages) and mean salinity by year (averaged over all sites).

each variable are shown in Figure 3. The overall  $r^2$  value for the size frequency analysis indicated that cluster membership explained 79.4% of the total variation in size frequencies between observations. Stepwise discriminant analysis indicated that salinity, *H. nelsoni* prevalence and *P. marinus* prevalence were significant ( $p < 0.10$ ) in explaining cluster membership, whereas depth, temperature, and the number of disease prevalence observations imputed were not significant explanatory variables (Table 1).

Cluster membership for many of the individual sites changed from year to year (Fig. 4). In 1990, a majority of sites classified into Cluster 1, a group with high mortality in the larger size classes, poor recruitment, a modal size of 77–81 mm, and low number of live oysters. Clusters with high spat densities contained only 3 sites, one in the St. Marys River (Cluster 6), and two in Tangier Sound (Cluster 5). Only 1 site, Piney Island Bar in Tangier Sound, was classified into Cluster 3, typified by relatively

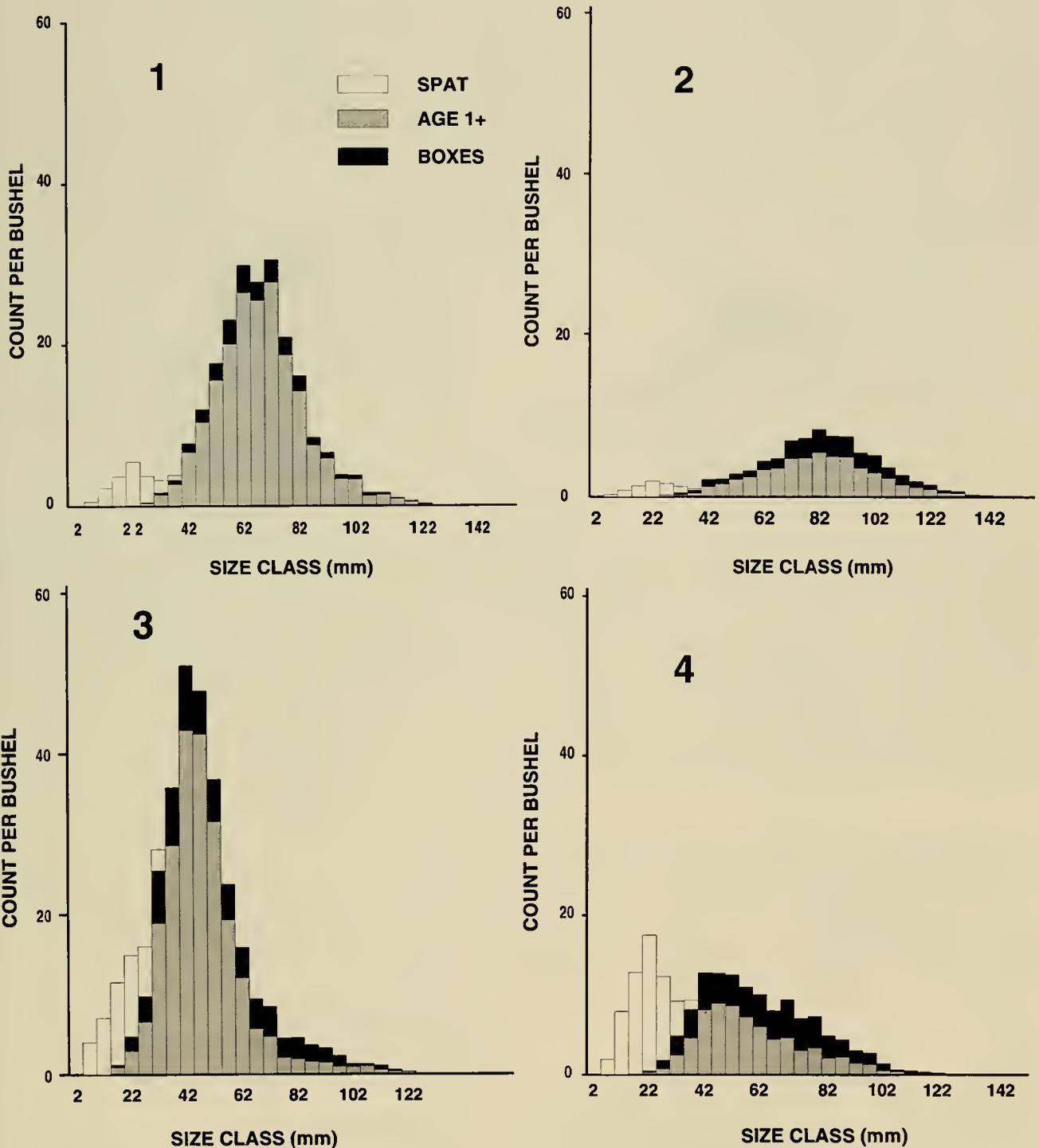


Figure 3. Mean population size structures for each of 6 clusters. Counts are based on counts per bushel of bottom materials collected by oyster dredge. Clusters are identified by bold numerals.

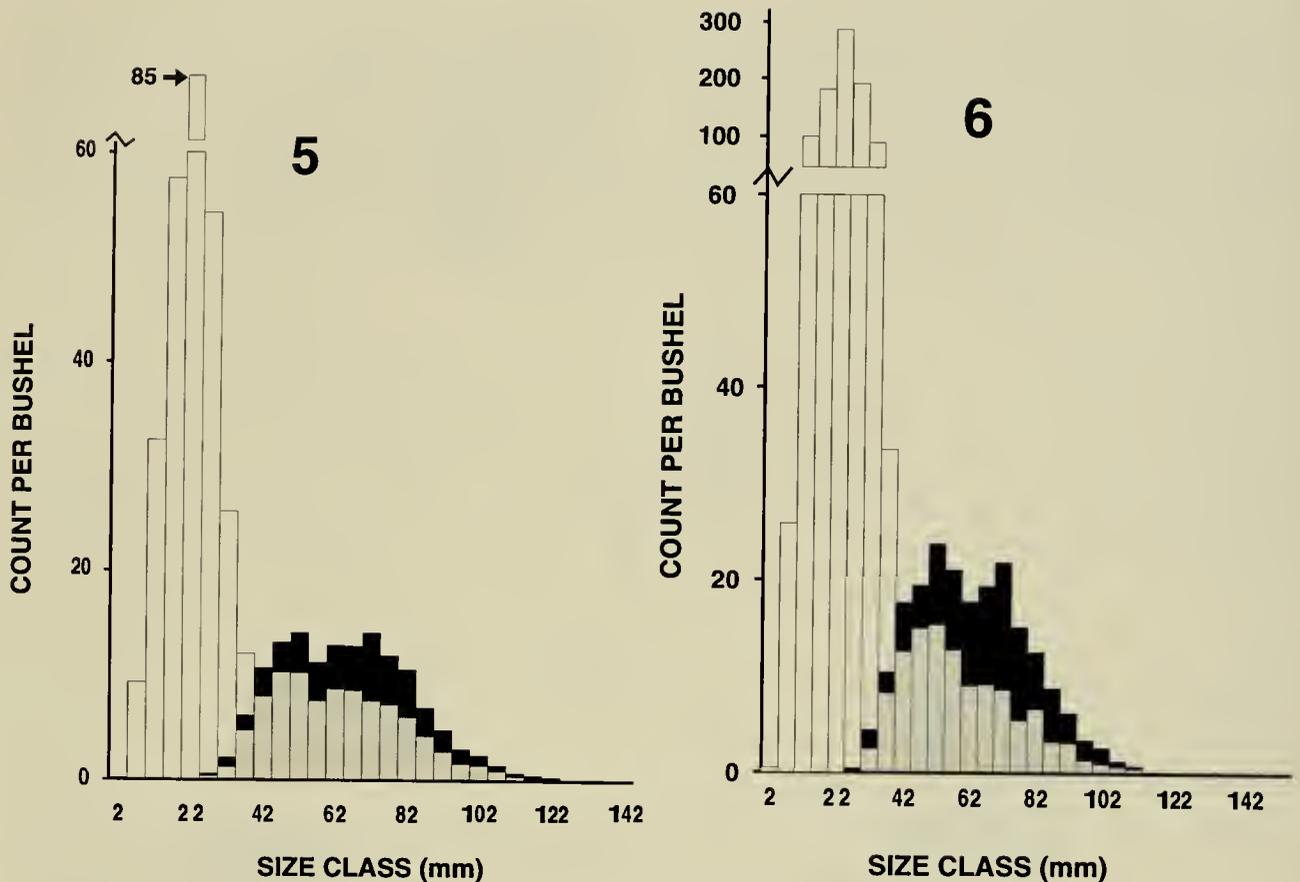


Figure 3. Continued.

high counts of small (age 1 + or 2 +) oysters. In 1991, a year with exceptionally high spatfall (Krantz 1992), 25 of 64 sites (39%) were classified into Cluster 5 or 6. Sixteen sites (25%) fell into Cluster 4, typified by moderate spatfall, small modal size, and high mortality of small oysters. Nearly all of the sites that classified into Clusters 1 and 2 (larger oysters with poor spatfall) were in lower salinity areas. Cluster 3 again contained only one site (Pagan Bar in the St. Marys River), in this case reflecting growth of the large set at this site in 1990. In 1992, due apparently to growth of the large 1991 year class, 45% of the sites were clas-

sified into Clusters 3 and 4 (the 2 northernmost sites in Cluster 3 did not have strong recruitment in 1991, but were planted with seed oysters from state seed areas in the previous spring). In 1992, both *P. marinus* and *H. nelsoni* infections reached unprecedented prevalence in Maryland (Krantz 1993); the high mortalities associated with this event are reflected in 67% of the sites being classified into either Cluster 2 (high mortality of larger oysters) or Cluster 4 (high mortality of smaller oysters). In 1993, a year with high spring runoff, generally low salinities, and very poor recruitment, all sites were classified into Clusters 1-4. The high mortality clusters (2 and 4) contained 72% of the sites. Most of the sites in the Tangier sound area, however, moved into Cluster 3 in 1993, with large numbers of small oysters and moderate mortality.

Only Cluster 1 typified what might be termed a harvestable oyster bar, because of relatively high densities of larger oysters (only oysters larger than 76 mm can be harvested legally in Maryland). Except in 1990, all of the sites in Cluster 1 were in the northernmost Bay or the upper reaches of the tributaries.

Sites where multiple samples were classified into different clusters are circled in Figure 4 (Holland Straights Bar in 1990 and Sandy Hill Bar in 1991). Two sites in 1990, and one in 1991 that were sampled twice did not differ in cluster membership between the 2 samples.

#### Population Attributes Analysis

Six clusters of observations also were derived from the population attributes analysis (Fig. 5). The overall  $r^2$  for this analysis

TABLE 1.

Summary of stepwise discriminant analysis to explain clustering of oyster population size structure.

Variable	Partial R <sup>2</sup>	F	p
Salinity	0.2256	13.635	0.0001
<i>H. nelsoni</i>	0.0889	4.549	0.0006
<i>P. marinus</i>	0.0415	2.007	0.0785
Temperature			ns
Depth			ns
Impute			ns

Clusters were the classes, with the variables listed as potential explanatory variables; p = probability of a greater F statistic under the null hypothesis of no effect of the variable on classification into clusters; ns = not significant at  $\alpha = 0.10$ ; impute = the number of imputed disease prevalence variables per observation (see text).

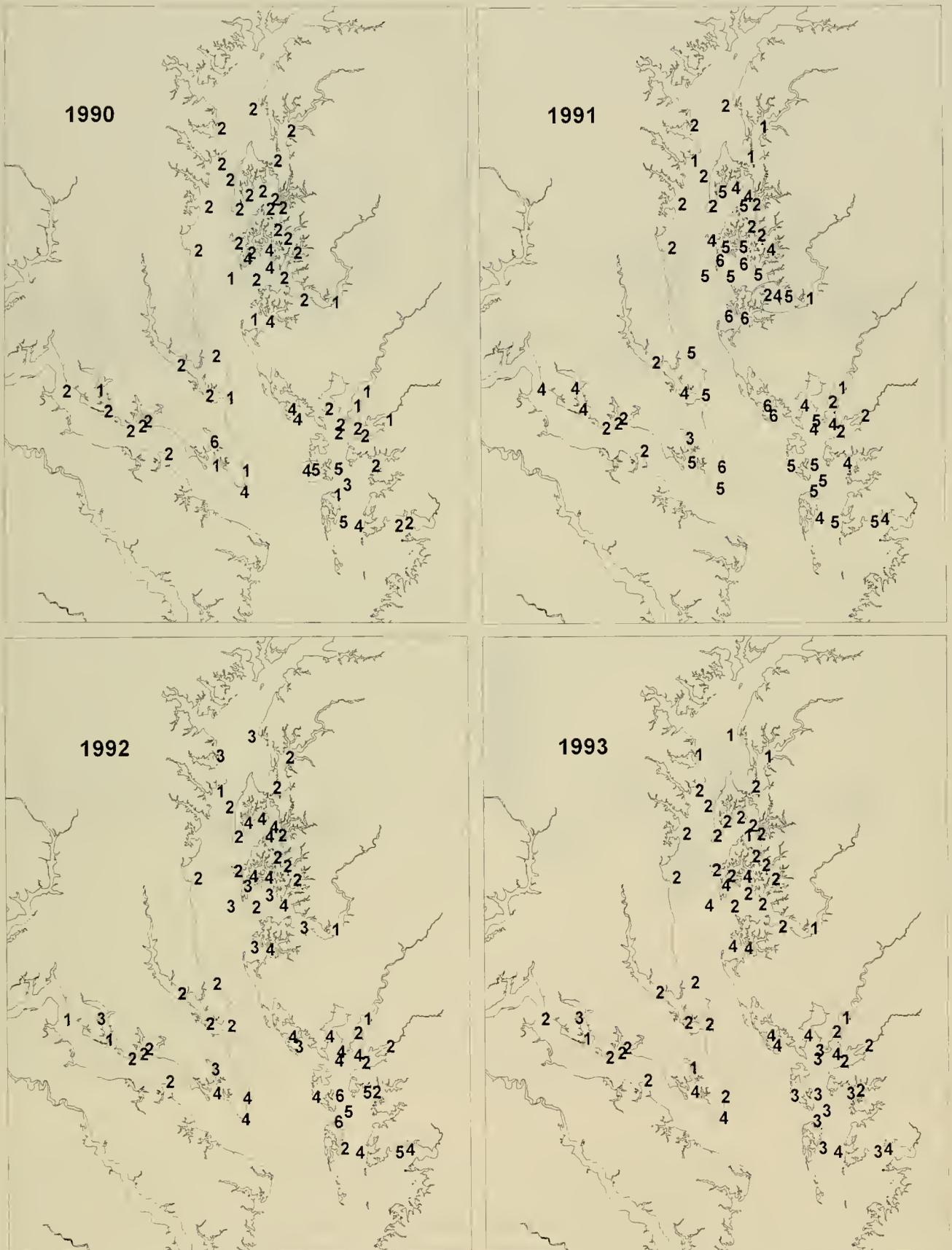


Figure 4. Size frequency analysis: geographical distribution of oyster bars by cluster for each year; numbers indicate cluster membership. Sites where multiple samples were classified into different clusters are circled.

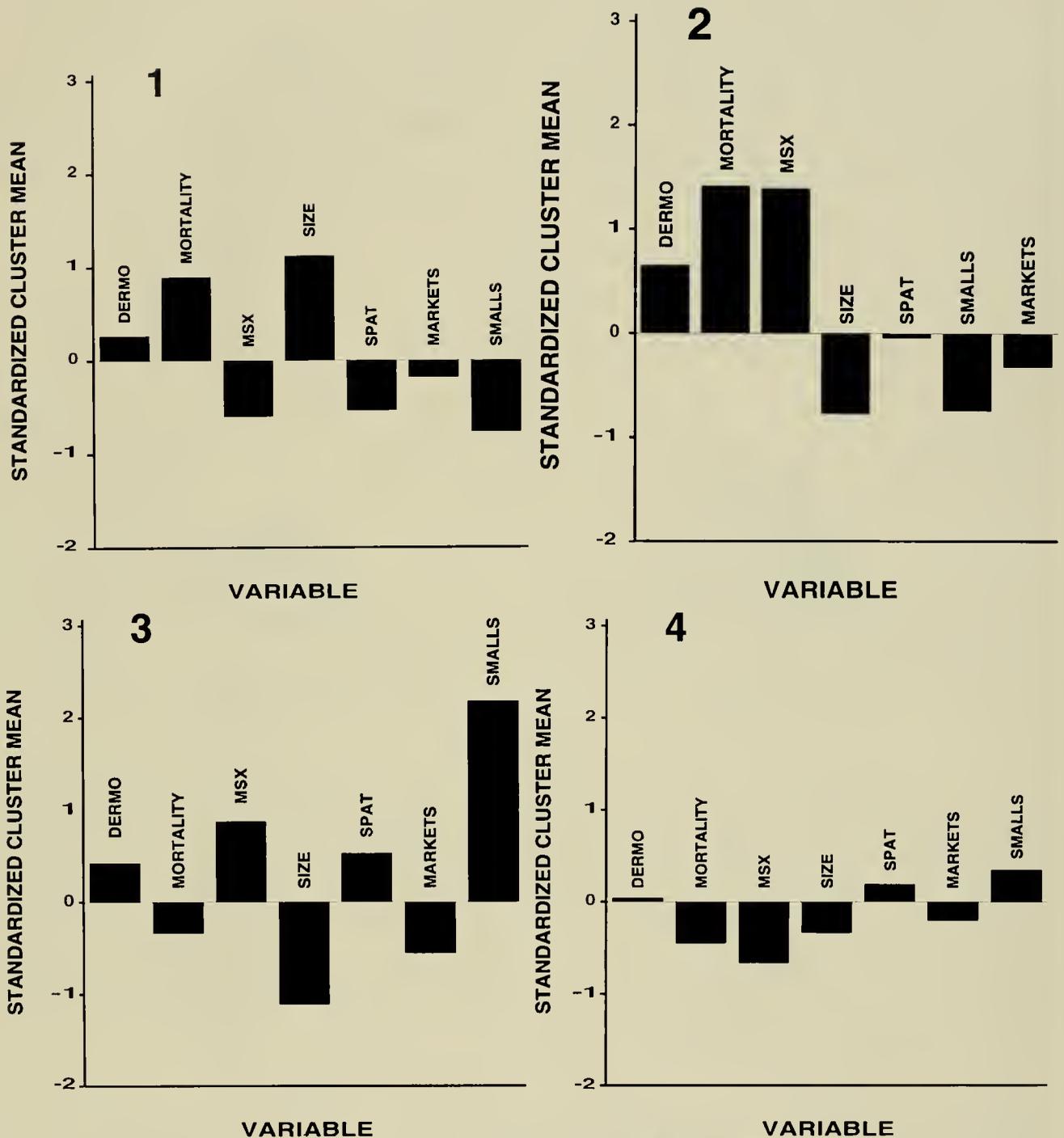


Figure 5. Means of variables for each cluster derived from the population attributes analysis, shown as relative deviations of the cluster means from the overall mean for each variable. Dermo = prevalence of *Perkinsus marinus* infections; MSX = prevalence of *Haplosporidium nelsoni* infections; spat = relative spat densities; markets = relative abundance of oysters >76 mm in shell height; smalls = relative abundance of oysters  $\leq$  76 mm in shell height. Clusters are identified by bold numerals.

indicated that 53.5% of the variation in the attributes between observations was explained by cluster membership. Cluster 1 was characterized by low counts of live oysters, low prevalence of *H. nelsoni*, large size of live oysters, poor recruitment, and moderately high mortality. Cluster 2, like the first, had low counts of live oysters, but with the highest prevalences of *P. marinus* and *H. nelsoni*, and highest mortality. Cluster 3 was dominated by high counts of small oysters, and above average recruitment, but also

high prevalence of *H. nelsoni*. Cluster 4 had below average prevalence of *H. nelsoni* and average prevalence of *P. marinus*, along with near-average counts of live oysters and spat. Cluster 5 had the highest counts of large oysters, with low mortality, low disease prevalence, and low recruitment. Cluster 6 had the lowest counts of market oysters, with moderate disease prevalence and mortality along with the highest recruitment.

Several spatial and temporal patterns were noted in the distri-

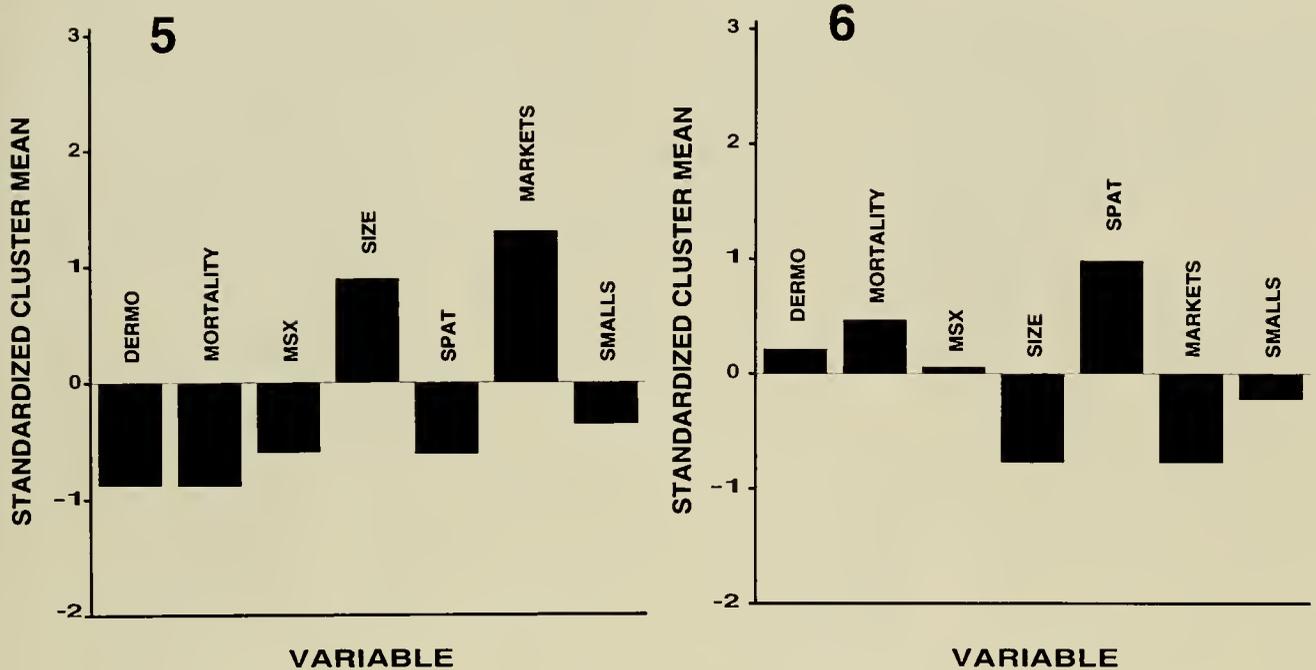


Figure 5. Continued.

bution of the population attributes clusters (Fig. 6). For Cluster 1, the number of sites increased over the 4 years, from 5% of the total in 1990 to 22% in 1993. The geographical distribution of Cluster 1 also expanded over the period. Cluster 2 (with the highest prevalence of *H. nelsoni* and highest mortality) contained only 1 site in 1990, 2 sites in 1991, and 3 sites in 1993, but in 1992, 23 sites (64%) were classified into this group. The highest prevalence and widest distribution of *H. nelsoni* ever recorded in Maryland were observed in 1992 (Krantz 1993). Cluster 3, with large counts of small oysters, occurred almost exclusively in 1) traditional seed production areas and 2) sites where large numbers of seed oysters had been planted by the state for growout and later harvest. Cluster 4, representing nearly average values of most of the attributes (except for low *H. nelsoni* prevalence) did not show clear spatial or temporal patterns. Cluster 5 was typified by high counts of large oysters, low disease prevalence, low mortality, and clear temporal and spatial trends, with 31 widespread sites (48%) in 1990, 15 sites (23%) in 1991, 7 sites (11%) in 1992, and 9 sites (14%) in 1993. In the later years, only sites with low to moderate salinity were classified into Cluster 5. Cluster 6 had the highest recruitment (as measured by annual spatfall); the expansion of this group in 1991 and its prevalence at Tangier Sound sites in 1992 reflected documented trends in spatfall (Krantz 1992, 1993).

#### DISCUSSION

The analyses reported here summarize many observations of a large group of variables associated with Maryland oyster populations over a 4-year period. The cluster analyses are not definitive, because there are many possible sets of assumptions that can result in somewhat different patterns of classification. However, it is informative to visualize the major properties of this large, complex data set in a few simple graphs and maps.

These classifications may be compared with a more traditional and subjective view. A biologist who has participated in the Maryland fall oyster survey for many years recognizes 4 general types

of oyster bars: 1) bars with sufficient recruitment to maintain population structure in spite of disease pressure; 2) bars with low, sporadic recruitment and unusual population structure, where disease impacts can be severe; 3) remnant bars where poor recruitment and disease pressure have left very low densities of old, slow-growing oysters; and 4) bars maintained by transplanting seed oysters (G. E. Krantz, pers. comm.). By inspection of the size frequency analysis (Fig. 3), it is difficult to identify the first type of population; perhaps Cluster 6, with the highest recruitment, is the best match, although densities of adult oysters are not high in this group. The second group, with unusual population structure, roughly matches Cluster 4, with low densities of small oysters, very high mortality and low recruitment. The third type, remnant bars, may be represented best by Cluster 2. This group has very low densities of fairly large oysters, with moderate recruitment (recruitment in this cluster probably was strongly influenced by the strong and widespread 1991 spatfall). The fourth group, bars maintained by transplanting seed oysters, could be represented by Clusters 1 and 3, both of which had relatively high densities of oysters, low mortality, and low recruitment. These 2 clusters differed mainly in the modal size of the oysters, perhaps reflecting plantings in the current year (Cluster 3) versus plantings in a previous year (Cluster 1). A review of the sites that clustered into these last categories suggests that although both clusters contained seeded bars, Cluster 1 also contained some low salinity natural bars that were not seeded. In 1993, Cluster 3 included several bars in Tangier Sound that had received substantial spat sets the previous year (Fig. 4).

*P. marinus* prevalence did not appear to have a major effect on the analysis of population attributes (note the small deviations, relative to the other variables, in Figure 5). This apparently counterintuitive result can be attributed to the fact that *P. marinus* infections were present at high prevalences at virtually all of the monitoring sites throughout the 4 years, and therefore this variable contributed less to classification. Also, this analysis probably was confounded by the fact that many observations had zero preva-

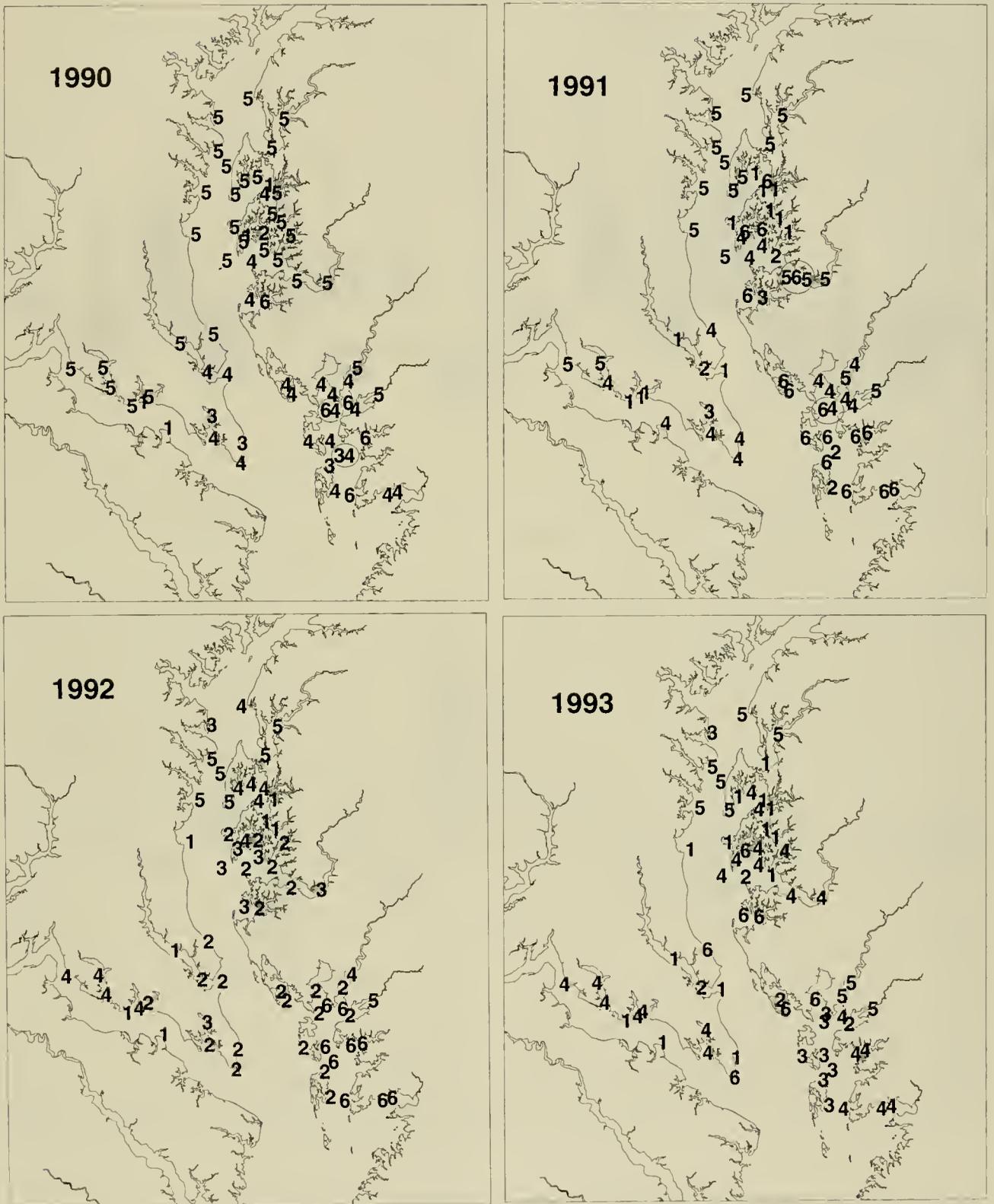


Figure 6. Population attributes analysis: geographical distribution of oyster bars by cluster for each year; numbers indicate cluster membership. Sites where multiple samples were classified into different clusters are circled.

lence of *H. nelsoni*. Several patterns observed in the population attributes analysis, however, highlight the dynamic nature of Maryland oyster populations in the early 1990s. As *P. marinus* and *H. nelsoni* expanded their range and increased in severity over

the period, the "typical" oyster bar profile (i.e., the most frequent clusters in Figure 6) changed from relatively high counts of market oysters and low disease prevalence (Cluster 5) to lower counts of smaller oysters and higher mortality (Cluster 4). This change can

be attributed mainly to the spread of *P. marinus* infections. Patterns associated with recruitment, growth, and movement of seed oysters also can be discerned in Figure 6.

Both views of oyster populations focus on recruitment and disease mortality as major determinants of abundance and size structure. Densities of spat and small oysters, both measures of recruitment, clearly had important effects on the cluster analyses. The discriminant analysis, however, indicated that *P. marinus* infections were significant in determining population structure based on size frequencies, along with *H. nelsoni* prevalences and salinity.

These analyses lead one to question what might have been the structure of natural oyster bars in the absence of epizootic diseases and fishing pressure (the latter variable could not be used in this analysis, because Maryland collects harvest statistics on a regional landings basis, rather than bar by bar). Unfortunately, oysters were not measured in surveys before 1990. Fishing mortality for 1990 and 1991 was estimated crudely from size frequency data by Smith and Jordan (1993) at 53% of harvestable oysters (>76 mm in shell height) on survey bars known to have been harvested in those years. Disease mortality between 1990 and 1993 varied from year to year, generally increasing over the period of study. Overall mean mortality was 31.2% for the 4 years. Background annual natural mortality at oyster bars not affected by *P. marinus* or *H. nelsoni* epizootics in the Choptank River estuary was slightly more than 3% (Christmas and Jordan 1991). These estimates cannot simply be combined to generate a profile of undisturbed oyster populations; for example, oyster bars that supported harvests had lower levels of parasitic infection prevalence and mortality than unharvested bars (Smith and Jordan 1993). The picture is complicated further because it can be assumed that a large percentage of oysters would continue to grow and reproduce almost indefinitely, when not subjected to harvest and diseases. In the absence of a detailed population model, one can only make the obvious supposition that without harvest and epizootics there would be substantially more oysters, and that they would be on average larger than in recent years.

The patterns of growth (inferred from the changing patterns seen in Figure 4) and recruitment observed over the period of study indicate that considerable potential for increased oyster abundance exists in Maryland's Chesapeake Bay. Any changes—whether due to climate, natural selection, or management intervention—that significantly reduced mortality from parasitic infections could result in large increases in the abundance of adult oysters. Recovery would be most rapid in areas that show strong recruitment in most years, but much of Maryland's oyster grounds could gradually be repopulated. Management efforts in recent years have been directed towards a strategy of restoring and maintaining substrate for spat settlement in selected higher salinity areas of the Bay (taking

advantage of and stimulating natural recruitment), and movement of substantial numbers of seed oysters to low salinity areas in the upper mainstem Bay and tributaries. Infections of *H. nelsoni* and the associated oyster mortalities generally do not occur in these low salinity areas, and although *P. marinus* infections do not disappear at lower salinities, mortality due to the latter parasite is thought to be delayed (Andrews 1988). These tactics have maintained a (much reduced) fishery, but populations of larger oysters have continued to decline Baywide.

The analyses reported here have suggested that statistical treatment of a suite of biological variables can provide a different and more informative view of oyster populations than classifications based on geographical regions or salinity. Both disease pressure and recruitment, key variables that structure these oyster populations, are associated with salinity, which in turn follows well-documented geographical trends in the northern Chesapeake Bay. But factors less closely associated with salinity, such as growth, oyster density, transplanting of seed oysters, and trends in overall population structure also were captured by the statistical models. For example, several of the oyster bars classified into Cluster 1 by the size frequency analysis (Figs. 3 and 4) were sites where harvestable populations were maintained by transplanting seed oysters. This work has demonstrated (qualitatively, at least) that salinity and geography alone are not sufficient surrogates for the dynamics of managed oyster populations.

Detailed mathematical modelling studies have been undertaken recently to simulate processes in oyster populations and parasite-host interactions over a range of environmental variation (Hofmann et al. 1992, Deksheniaks et al. 1993). These studies should be extended to explore the potential for optimizing management of oyster populations in the presence of both *P. marinus* and *H. nelsoni* under conditions endemic to Chesapeake Bay. It is unlikely that the complex interactions of recruitment dynamics, parasites and hosts, and environmental factors can be captured fully by intuitive or statistical approaches.

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## IN VITRO SPORULATION OF THE CLAM PATHOGEN *PERKINSUS ATLANTICUS* (APICOMPLEXA, PERKINSEA) UNDER VARIOUS ENVIRONMENTAL CONDITIONS

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**ABSTRACT** *Perkinsus atlanticus* Azevedo (1989) is a protozoan parasite of clams *Tapes decussatus* and has been responsible of extensive mortalities in clams breeding areas located on the South coast of Portugal, Algarve. Its developmental cycle was described *in vitro*, using artificial zoosporulation techniques, and allow construction of a proposed life cycle associated with the host clam *T. decussatus*. The study of various environmental factors on *P. atlanticus* zoosporulation allowed for the determination of the optimal range of conditions for parasite development, i.e., 24–28°C temperature, 25–35‰ salinity, and pH 7–8. These range of values are in accordance with those measured in infected cultured clams areas.

**KEY WORDS:** *Perkinsus atlanticus*, Apicomplexa: Perkinsea, *Tapes decussatus*, Mollusca: Bivalvia, *in vitro* study, sporulation, environmental factors

### INTRODUCTION

Sporozoans of the genus *Perkinsus* are known to parasitize marine molluscs and have been associated with massive mortalities in cultured and wild populations throughout the world. *Perkinsus marinus* infects the American oyster *Crassostrea virginica* along the east coast of the United States (Mackin et al. 1950, Perkins and Menzel 1966, 1967, Perkins 1976, 1988) and the Gulf of Mexico (Andrews and Ray 1988). *Perkinsus olseni* has been associated with mortality in abalone *Haliotis ruber* from Australia (Lester 1980, Goggin and Lester 1987). In Canada, a new species *Perkinsus karlsoni* was recently described in scallops *Argopecten irradians* (McGladerry et al. 1991). In Portugal, *Perkinsus atlanticus* parasites are associated with seasonal mortalities in cultured clams *Tapes decussatus* (Ruano 1985, Azevedo 1989). Vegetative stages of parasites, i.e., trophozoites, proliferate by schizogony in gill tissues as well as mantle and digestive gland, causing respiratory failure and death of the host.

Several studies have been made on *P. marinus*, and its life cycle is now well known (Perkins and Menzel 1966, 1967, Perkins 1976, 1988). *In vitro* culture of *P. marinus* under different environmental conditions demonstrated that optimal values for prezoosporangia sporulation were between 18 and 28°C and above 6‰ salinities (Chu and Greene 1989). Research on the tolerance of several *Perkinsus* spp. to various environmental factors revealed that prezoosporangia and trophozoites are highly resistant to a range of temperatures, salinities and chlorine (Goggin et al., 1990). No studies on the effects of pH and dissolved oxygen on *Perkinsus* spp. development have been reported. For *P. atlanticus*, histological and ultrastructural studies have been published (Chagot et al. 1987, Azevedo 1989, Azevedo et al. 1990).

In this work, *in vitro* sporulation of *P. atlanticus* was studied;

our results supplement current knowledge on *Perkinsus* protozoans and allow construction of a proposed life cycle associated with the host clam *T. decussatus*. A range of temperatures, salinities, pH and dissolved oxygen was tested and allowed us to determine optimal conditions for *P. atlanticus* sporulation, and hence predict conditions favorable for spread in clam culture.

### MATERIALS AND METHODS

*P. atlanticus* parasites were obtained from infected, cultured clams *T. decussatus* collected in the Ria Formosa, Algarve, an area intensively parasitized in Portugal. The animals were maintained at the laboratory in a closed circulation system with aerated seawater (24 ± 1°C temperature, 30 ± 1‰ salinity). Circulation water was passed through a biofilter and sand filter and the clams were fed with *Tetraselmis* sp. algae.

#### *In Vitro* Culture of *Perkinsus atlanticus*

Gills were incubated in fluid thioglycollate medium (FTM, Difco) for 3 to 4 days at 20°C in the dark; infections were detected using Lugol's iodine solution (Ray 1952, 1966). After removal from the FTM, infected tissues were washed 3 times in sterile seawater containing 50 units/mL nystatin, 200 units/mL penicillin and 200 units/mL streptomycin. Tissues were then fragmented and dissociated during 20 min in a saline solution supplemented with 0.05% trypsin, 0.125% EDTA and 1 mg/mL glucose at pH 7.2. Dissociation was stopped by addition of 10% foetal calf serum. The suspension was filtered through a 90 µm sieve. Parasitic cells were isolated from gill cells by serial centrifugation and counted using an inverted microscope with hemocytometer. Prezoosporangia were then cultured in Petri dishes containing 15 mL of 30‰ salinity seawater supplemented with 0.1 mg/L penicillin-streptomycin and 4000 U/mL nystatin. Petri dishes were incubated in the dark at 24°C and examined daily under an inverted phase contrast microscope (Nikon MTD 200×).

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*Effect of Temperature, Salinity, pH and Dissolved O<sub>2</sub> on Perkinsus atlanticus Sporulation*

Parasites were purified according to the protocol described above. Two Petri dishes with prezoosporangia were prepared for each value of the 4 parameters tested (temperature, salinity, pH, dissolved oxygen).

- Temperature tested values (7, 15, 24 and 28°C) were controlled using incubators.
- Salinity tested values were 5, 10, 15, 25, 30 and 35‰. Purification of prezoosporangia was accomplished using 30‰ sea water for the 35, 30 and 25 values and with 15‰ sea water for the 5, 10 and 15 values in order to avoid osmotic shocks.
- pH values tested (4, 5, 6, 7 and 8) were adjusted using, respectively, N/5 HCl, citrate-phosphate buffer, phosphate buffer, carbonate-bicarbonate buffer or N/10 NaOH. Values were monitored daily with a pHmeter (Metrohm Herisau-E-588).
- Dissolved oxygen (DO) tested values (i.e., 9.2, 5.8, and 2.2 mg/mL) were obtained by bubbling air or nitrogen in sea water and measured with an oxygen monitor (Nester instruments). Prezoosporangia were cultivated in Leighton tubes.

The cultures were placed in the dark and examined daily under an inverted phase contrast microscope for 5 days. The number of cells, in each stage of development, was counted in 4 to 7 areas per plate. The evaluation of *P. atlanticus* sporulation was made using the following criteria: beginning of sporulation (days), sporulation and viability rates (on day 4) and appearance of free zoospores (days). Viability of live cells (prezoosporangia and sporulating cells) was determined by their appearance in contrast with dead cells which showed collapsed, thickened or deformed cell walls and contain large lipid droplets or dark irregular areas. After the discharge tube was formed, viable sporangia were considered to be committed to sporulation.

## RESULTS

### *In Vitro Culture of Perkinsus atlanticus Prezoosporangia*

During incubation in FTM, parasitic cells increased in size, resulting in prezoosporangia which appear as symmetrically round black spheres when coloured with Lugol's iodine solution (Fig. 1). After purification, the resulting pellet consisted of numerous spherical cells, 25 to 100 µm in diameter, with a large vacuole and a peripheral ring of granular cytoplasm.

Different stages of *P. atlanticus* sporulation are shown in Figure 2. From the first day in seawater, prezoosporangia adhere to the bottom of the Petri dishes; the vacuole progressively decreases in volume and the cytoplasm becomes granular (Fig. 2a). On the second day, a pore is formed in the cell wall followed by formation of a discharge tube (Fig. 2b). The cytoplasm contracts, releasing intravacuolar fluid to the space between the cell wall and the protoplast, and moves into the center (Fig. 2c) where it undergoes the first binary division (Fig. 2d). Successive bipartitions give rise to intermediate stages with 4 (Fig. 2e), 8, 16 (Fig. 2f), 32, 64 (Fig. 2g), and more cells until there are hundreds of biflagellated zoospores inside each sporangium (Fig. 2h). When mature, 2 to 3 days after the beginning of sporulation, zoospores swim into the sea water through the discharge tube following breakage of a plug of wall material located in the pore at the base of tube (Fig. 2i). This has been previously reported by Azevedo et al. (1990) and in related species (*P. marinus*, Perkins and Menzel 1966, 1967).

In our controlled conditions (30‰ salinity, 24°C temperature), sporulation of *P. atlanticus* occurs *in vitro* within 2 to 3 days. Prezoosporangia formation leads to the development of hundreds of zoospores, which are an infective form of the parasite (Perkins 1976). Our results combined with previously published data (Azevedo 1989, Azevedo et al. 1990) allowed us to describe the main stages of the *P. atlanticus* life cycle (Fig. 3).

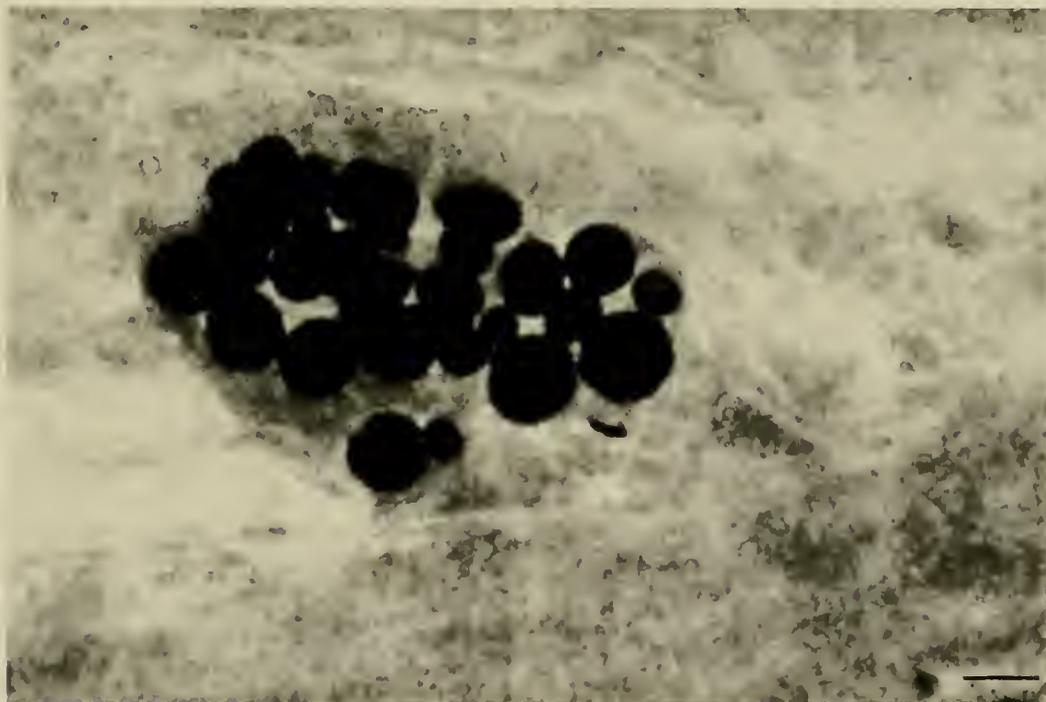


Figure 1. *P. atlanticus* prezoosporangia in clam gill tissues coloured with Lugol iodine solution. Scale-bar = 100 µm.

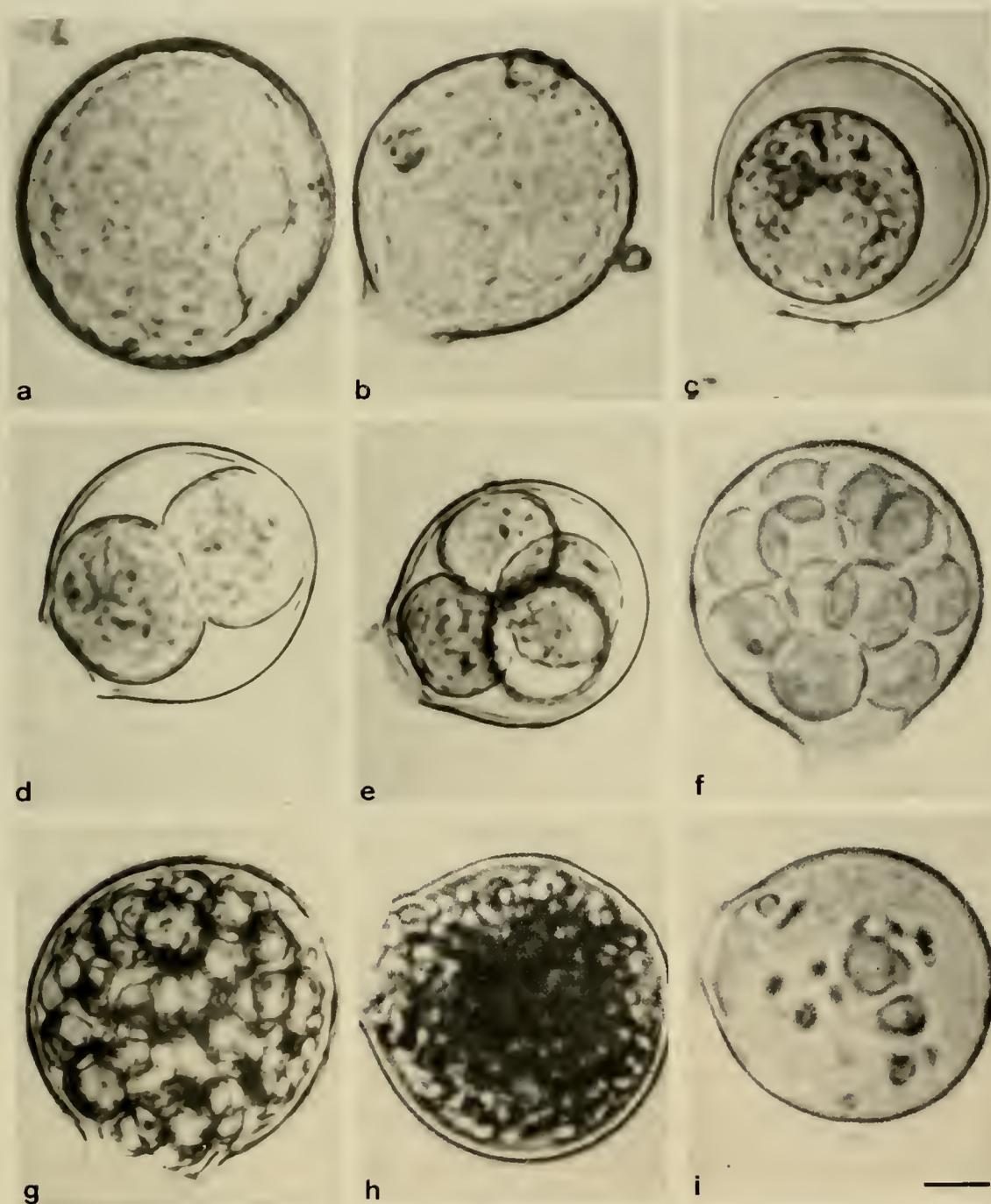


Figure 2. *In vitro* sporulation of *P. atlanticus* in seawater (phase contrast). a, beginning of eccentric vacuole subdivision; b, formation of a discharge tube; c, cytoplasm contraction; d, 2-cell stage; e, 4-cell stage; f, 16-cell stage; g, 64-cell stage; h, stage with hundreds of motile zoospores; i, zoospore release. Scale-bar = 20  $\mu\text{m}$ .

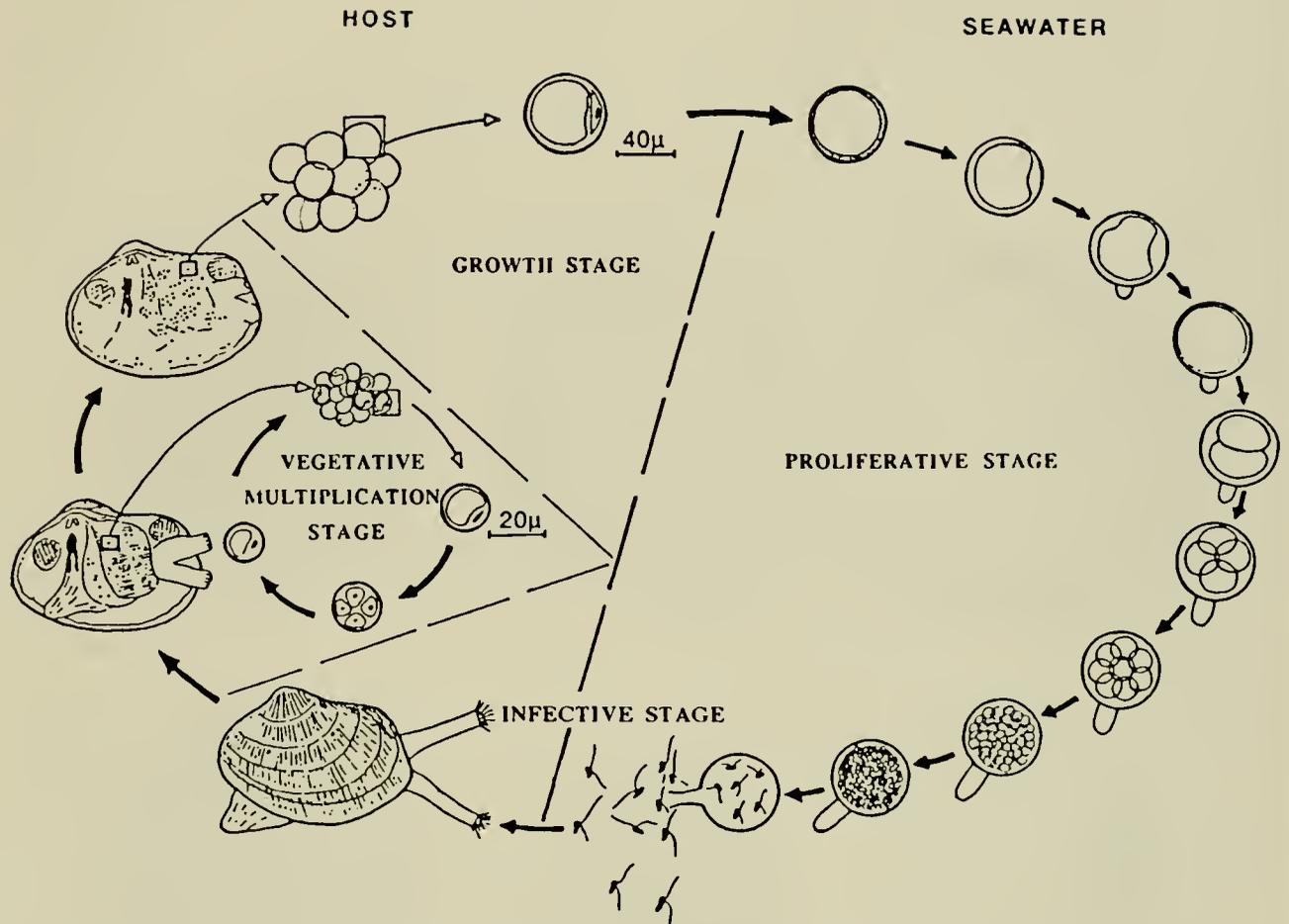


Figure 3. Schematic life cycle of *P. atlanticus*, parasite of clams *T. decussatus*.

- *Vegetative multiplication stage* in live host tissue, which consists of multiplication of trophozoites (schizogony), inducing a host cellular defense response which results in the formation of cysts and granulomas (Chagot et al. 1987, Montes et al. 1995).
- *Growth stage* when the host dies, creating anaerobic conditions favourable to the transformation of trophozoites into prezoosporangia. According to our observations, we assume that moribond tissues provide anaerobic conditions similar to those found in FTM. However, some studies on related species have shown development into prezoospores and sporulation of the pathogen, without exposure to FTM (Kleinschuster et al. 1994).
- *Proliferative stage* in sea water where prezoosporangia become mature and undergo successive bipartitions of the protoplast (sporulation) resulting in the release of hundreds of zoospores through a discharge tube.
- *Infective stage* when free zoospores infect other clams. Healthy clams are probably infected during the filtration of sea water. Controlled infection experiments demonstrated the capacity of *P. atlanticus* zoospores to infect healthy clams without an intermediate host (Azevedo 1990, Vigario et al., unpubl. data). Ultrastructural studies on *Perkinsus* spp. zoospores described an apical complex which could be involved in host cell penetration (Perkins 1976, Azevedo 1989).

TABLE 1.

*In vitro* development of *Perkinsus atlanticus* sporangia at different temperatures.

Temperature (°C)	Beginning of Sporulation (Days)	Appearance of Free Zoospores (Days)	Day 4		
			Viability (%)	Sporulation (%)	Total Cell Count
7	—	—	49%	0%	65
15	—	—	53%	0%	75
24	1–2	2–3	62%	90%	95
28	1–2	2–3	55%	82%	60

TABLE 2.

*In vitro* development of *Perkinsus atlanticus* sporangia at 24°C after 5 and 14 days of culture at 7 and 15°C.

Temperature (°C)	Beginning of Sporulation (Days)	Appearance of Free Zoospores (Days)
7	1-2	2-3
15	1-2	2-3

#### Effect of Temperature, Salinity, pH and Dissolved Oxygen on *Perkinsus atlanticus* Sporulation

##### Temperature

Results concerning the effects of temperature on *P. atlanticus* sporulation are reported in Table 1. Sporulation occurred only at 24 and 28°C, beginning between the first and second day and finishing between the second and third day of culture. At 7 and 15°C, sporulation did not occur, but the viability of the sporangia was not affected. When transferred to 24°C after 5 and 15 days, sporulation began within 1 or 2 days after and zoospores appeared within 2 to 3 days (Table 2).

##### Salinity

Results concerning the effects of salinity on *P. atlanticus* sporulation are reported in Table 3. Salinities ranging between 25 and 35‰ promoted the best development of parasitic cells; at these salinities zoospores appeared within the second and third day and sporulation rates remained close to 90%. In 10 and 15‰ salinity, sporulation began later and zoospores appeared only the fourth and fifth day. Viability and sporulation rates also decreased with salinity. No sporulation occurred within 5 days at 5‰.

##### pH

Results on the effects of pH on *P. atlanticus* sporulation are reported in Table 4. Sporulation was initiated at all pH values, starting within 1 and 2 days, but zoospores did not appear after 5 days at pH values under 7. Viability rates were similar for all pH values tested, whereas sporulation rates were significantly lower at the lowest pH's.

##### Dissolved Oxygen

Results concerning the effects of DO on *P. atlanticus* sporulation are reported in Table 5. In spite of the DO decrease in all tubes during the experiment, sporulation occurred at all DO val-

ues. Zoospores appeared later at the lowest value in spite of viability and sporulation rates observed.

## DISCUSSION

*In vitro* culture of *P. atlanticus* resulted in zoosporulation producing hundreds of free motile zoospores in sea water, as previously described by Azevedo et al. (1990). Our results supplement current knowledge on *Perkinsus* protozoans and allow construction of a proposed life cycle associated with the host clam *T. decussatus*: like other species of the genus, it consists of 4 principal stages, the first two occur inside the hosts tissues, and the other 2 stages occur in seawater. We assume that the growth stage, obtained in artificial conditions in fluid thioglycollate medium, happens in natural conditions when the host dies, by means of anaerobic conditions created inside tissues, as suggested by previous authors (Ray 1966, Perkins et al. 1966, 1967). When released in seawater, *P. atlanticus* sporangia sporulate quickly and produce in 2 to 3 days hundreds of biflagellated zoospores which are an infective and dispersive stage of the parasite. There is no evidence for the presence of an intermediate host during the infection process involving *P. atlanticus*. A carrier has been reported in the transmission of *P. marinus* (White et al. 1987).

Our experiments to determine the effects of environmental factors on *P. atlanticus* sporulation indicate that the optimal range of conditions for parasite development: 24–28°C temperature, 25–35‰ salinity, pH 7–8. Results for dissolved O<sub>2</sub> must be regarded as tentative due to the significant drop in dissolved oxygen which occurred during the experiment. Chu and Greene (1989) reported that *P. marinus* sporangia could only withstand temperatures as low as 4°C for less than 4 days and that optimal sporulation occurred at 28°C and salinities between 10 and 34‰. Induced sporulation of *P. atlanticus* sporangia was reported at 25‰ salinity and 28°C temperature (Azevedo et al. 1990).

The range of temperatures and salinities for *P. atlanticus* sporulation determined in this study specifies those previously reported for other species and confirms the tolerance of this genus towards environmental factors (Chu and Greene 1989, Goggin et al. 1990). Below the optimal values mentioned above, *P. atlanticus* sporangia either die or stop their development. For example, parasites do not sporulate below 15°C and begin sporulation when transferred to 24°C. In view of the low mortality rates observed *in vitro* below the optimal temperature values, *P. atlanticus* may stop its development when conditions become unfavourable (wintertime) and sporulate when they become better (summertime). In the same way, *P. atlanticus* sporangia may withstand the water freshening during winter and sporulate when salinity increases. In the

TABLE 3.

*In vitro* development of *Perkinsus atlanticus* sporangia at different salinities.

Salinity (‰)	Beginning of Sporulation (Days)	Appearance of Free Zoospores (Days)	Day 4		
			Viability (%)	Sporulation (%)	Total Cell Count
5	—	—	25%	0%	77
10	3-4	4-5	28%	38%	93
15	2-3	4-5	20%	71%	86
25	1-2	2-3	53%	90%	79
30	1-2	2-3	64%	92%	77
35	1-2	2-3	53%	90%	57

TABLE 4.  
In vitro development of *Perkinsus atlanticus* sporangia at different pH values.

pH	Beginning of Sporulation (Days)	Appearance of Free Zoospores (Days)	Day 4		
			Viability (%)	Initiation of Sporulation (%)	Total Cell Count
4	1-2	-	82%	52%	82
5	1-2	-	85%	35%	73
6	1-2	-	79%	76%	90
7	1-2	2-3	74%	70%	58
8	1-2	2-3	85%	69%	80

TABLE 5.  
In vitro development of *Perkinsus atlanticus* sporangia at different DO values.

DO Initial (mg/ml)	DO after 4 Days (mg/ml)	Appearance of Free Zoospores (Days)	Day 4		
			Viability (%)	Sporulation (%)	Total Cell Count
9.2	2.8	2-3	71	89	94
5.8	1.5	2-3	64	89	73
2.2	0.4	3-4	69	75	79

Ria Formosa, Portugal, environmental factors in clam culture beds range between 10–30°C temperature, 25–35‰ salinity, 7.5–8.5 pH and 5.8–18.1 mg/mL dissolved oxygen, minimum values occurring in winter and maximum in summer (Falcao 1988). According to the optimal range of conditions determined in this work, *P. atlanticus* parasites seem to have, in summer, the ideal conditions for their development and proliferation. This is confirmed by the observation that the highest levels of parasitic infection in cultured clams occur during summertime (Ruano 1989). These results may contribute to the development of management strate-

gies to reduce the disease dispersal and the pathogen impact on clam breeding areas.

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## DIFFERENCES IN LECTIN-BINDING BY HEMOCYTES OF OYSTERS (*CRASSOSTREA VIRGINICA*) FROM THREE REGIONS AND FURTHER EVIDENCE FOR THE CORRELATION BETWEEN THE PRESENCE OF LATHYROSE AND THE ABSENCE OF *HAPLOSPORIDIUM NELSONI*

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**ABSTRACT** Hemocytes collected from *Crassostrea virginica* (Gmelin) originating from Malpeque Bay, Canada, Galveston Bay, TX, and Church Creek, Johns Island, SC, were exposed to serial dilutions of 9 lectins and the number of agglutinated cells/100 cells was ascertained at each dilution. Also, saccharidal residues known to inhibit the agglutination of lectin-treated cells were employed to test their effectiveness. Among these, N-acetyl-D-glucosamine, D(+)-glucose, and D(+)-mannose, which usually are specific inhibitors of cell clumping mediated by the *Lathyrus odoratus* lectin did not have this effect. This confirms earlier studies. Hence, the nature of the hemocyte surface saccharide, designated as lathyrose for convenience, that binds to the *L. odoratus* lectin remains unknown. Earlier studies had suggested that the presence of lathyrose on the hemocyte surfaces of *C. virginica* may signal innate resistance to *Haplosporidium nelsoni* (Cheng et al. 1994). The results presented herein further support this hypothesis as the percentage of lathyrose-positive hemocytes was significantly higher in oysters from Malpeque Bay and Galveston Bay where *H. nelsoni* has never been reported than in those from Church Creek where preliminary data indicate that this pathogen occurs in approximately 4% of the oysters. There were some qualitative and quantitative differences in the binding affinities of certain lectins to hemocytes from the 3 regions. These probably reflect strain and seasonal differences.

**KEY WORDS:** Lectins, oyster hemocytes, *Crassostrea virginica*, *Haplosporidium nelsoni*, *Lathyrus odoratus*

### INTRODUCTION

It was reported earlier (Cheng et al. 1993) that by employing 8 lectins and inhibiting the agglutination of hemocytes mediated by these molecules by use of specific sugar residues, both qualitative and quantitative differences occurred between the binding affinities of the cells from oysters, *Crassostrea virginica*, from Apalachicola Bay, FL, and Galveston Bay, TX. These differences were attributed to strain differences. As the identification of surface saccharides by use of lectins on oyster hemocytes may provide insights into how these cells recognize self from nonself (Mullainadhan and Renwartz 1986, and others), the study being reported herein in part represents an extension of the earlier one.

Also, it was reported earlier (Cheng et al. 1994) that there is a saccharide on the surface of hemocytes of *C. virginica* that may serve as a marker for innate resistance to the protozoan pathogen *Haplosporidium nelsoni* (MSX). For convenience, this yet uncharacterized saccharide has been designated as "lathyrose" (Cheng and Dougherty 1994). To further test the existence of a possible correlation between the presence of lathyrose and resistance to *H. nelsoni*, the presence of this saccharide on hemocytes of *C. virginica* from 3 additional regions were investigated. *H. nelsoni* has never been reported in two of these while this pathogen occurs in a small percentage of the oysters from the third site.

### MATERIALS AND METHODS

#### Oysters

The non-South Carolina oysters, *C. virginica*, employed in this study were obtained from a local commercial source but originated

from 2 regions: Malpeque Bay, Canada, and Galveston Bay, TX. The origins of these oysters are documented. *H. nelsoni* has not been reported from either region (S. McGladdery, pers. comm., J. Couch, pers. comm.). The third group of oysters was harvested from Church Creek, Johns Island, SC, where *H. nelsoni* occurs in approximately 4% of 50 oysters sampled during the period when the collections were made (Cheng, unpubl.). All of the oysters were collected between the last week in January and the first week in March, 1995. These were maintained in running seawater (28–30‰ salinity) at 10–15°C until bled. None was maintained for more than 2 weeks. The numbers of Malpeque Bay, Galveston Bay, and Church Creek oysters the hemocytes from which were exposed to the lectins are presented in Table 1.

#### Hemocyte Collection

Approximately 2 mL of whole hemolymph were collected from the adductor muscle sinus of each oyster by use of a sterile 21-gauge needle and a 1-mL tuberculin syringe. The samples were washed 3 times in isotonic (540 mOs) saline (IS) involving centrifugation at 300g. After the third wash, the cell pellets were gently resuspended in 2 mL of IS. The final cell counts averaged  $1-2 \times 10^4$ /mL.

#### Lectins

A total of 9 purified lectins were tested against the hemocyte samples from oysters from the 3 regions: *Lathyrus odoratus* (sweet pea), *Limulus polyphemus* (horseshoe crab, limulin), *Tetragonolo-*

TABLE 1.

Mean numbers of agglutinated hemocytes/100 cells  $\pm$  SDs from *Crassostrea virginica* harvested from 3 regions and exposed to 5 concentrations of 9 lectins.

Lectin Origin of Oysters	Lectin Concentrations				
	1:1	1:64	1:512	1:1024	1:2048
<i>Lathyrus odoratus</i>					
Malpeque Bay (n = 22)	61.5 $\pm$ 4.8	17.6 $\pm$ 6.3	4.8 $\pm$ 0.1	0	0
Galveston Bay (n = 23)	68.1 $\pm$ 0.6	20.6 $\pm$ 6.9	10.7 $\pm$ 6.3	10.3 $\pm$ 10.7	0
Church Creek (n = 27)	43.8 $\pm$ 2.4	12.8 $\pm$ 4.3	6.6 $\pm$ 5.5	5.4 $\pm$ 3.4	0
<i>Limulus polyphemus</i>					
Malpeque Bay (n = 18)	18.6 $\pm$ 9.5	9.1 $\pm$ 9.4	7.8 $\pm$ 6.8	6.8 $\pm$ 4.5	4.8 $\pm$ 3.6
Galveston Bay (n = 15)	32.2 $\pm$ 16.7	21.7 $\pm$ 6.9	9.0 $\pm$ 1.2	4.1 $\pm$ 0	2.6 $\pm$ 0.4
Church Creek (n = 25)	20.1 $\pm$ 7.7	9.2 $\pm$ 4.0	4.9 $\pm$ 5.5	4.4 $\pm$ 3.5	0
<i>Tetragonolobus purpureas</i>					
Malpeque Bay (n = 22)	23.4 $\pm$ 11.6	16.1 $\pm$ 10.7	12.8 $\pm$ 5.8	9.1 $\pm$ 4.0	0
Galveston Bay (n = 20)	24.7 $\pm$ 14.0	9.3 $\pm$ 8.5	6.4 $\pm$ 3.3	3.3 $\pm$ 1.0	0
Church Creek (n = 16)	18.4 $\pm$ 8.3	7.8 $\pm$ 3.6	6.1 $\pm$ 3.5	5.6 $\pm$ 2.3	3.0 $\pm$ 3.2
<i>Dolichos biflorus</i>					
Malpeque Bay (n = 14)	22.1 $\pm$ 13.0	16.2 $\pm$ 11.1	9.4 $\pm$ 8.5	5.7 $\pm$ 5.0	0
Galveston Bay (n = 12)	30.1 $\pm$ 13.5	17.9 $\pm$ 7.9	10.4 $\pm$ 1.2	0	0
Church Creek (n = 16)	25.8 $\pm$ 10.5	13.3 $\pm$ 9.0	7.4 $\pm$ 5.2	6.9 $\pm$ 4.5	4.7 $\pm$ 3.8
<i>Glycine max</i>					
Malpeque Bay (n = 15)	15.2 $\pm$ 7.2	7.0 $\pm$ 1.4	6.5 $\pm$ 3.6	5.0 $\pm$ 3.3	0
Galveston Bay (n = 15)	27.8 $\pm$ 6.3	3.8 $\pm$ 0.2	0	0	0
Church Creek (n = 17)	19.3 $\pm$ 8.5	10.8 $\pm$ 7.0	8.6 $\pm$ 5.4	3.8 $\pm$ 1.4	0
<i>Arachis hypogaea</i>					
Malpeque Bay (n = 15)	23.2 $\pm$ 10.8	11.6 $\pm$ 8.9	10.4 $\pm$ 5.6	4.3 $\pm$ 2.1	0
Galveston Bay (n = 11)	29.2 $\pm$ 13.5	15.9 $\pm$ 14.4	7.1 $\pm$ 6.1	0	0
Church Creek (n = 17)	21.9 $\pm$ 9.0	11.2 $\pm$ 8.6	5.1 $\pm$ 4.7	3.4 $\pm$ 1.7	0
<i>Sambucus nigra</i>					
Malpeque Bay (n = 20)	9.7 $\pm$ 5.6	5.3 $\pm$ 2.9	4.2 $\pm$ 2.6	0	0
Galveston Bay (n = 15)	18.3 $\pm$ 0.4	8.4 $\pm$ 4.8	0	0	0
Church Creek (n = 15)	12.3 $\pm$ 4.9	6.6 $\pm$ 3.2	4.7 $\pm$ 1.1	3.0 $\pm$ 1.8	0
<i>Triticum vulgare</i>					
Malpeque Bay (n = 17)	26.5 $\pm$ 10.3	10.1 $\pm$ 5.5	9.1 $\pm$ 3.3	4.7 $\pm$ 1.7	0
Galveston Bay (n = 22)	27.7 $\pm$ 8.7	16.9 $\pm$ 9.0	8.0 $\pm$ 2.3	0	0
Church Creek (n = 18)	27.5 $\pm$ 9.9	13.7 $\pm$ 9.2	3.9 $\pm$ 2.2	2.7 $\pm$ 1.8	0
Con A					
Malpeque Bay (n = 28)	58.3 $\pm$ 7.4	12.9 $\pm$ 5.1	5.3 $\pm$ 2.7	0	0
Galveston Bay (n = 25)	56.2 $\pm$ 1.0	16.5 $\pm$ 2.0	7.7 $\pm$ 3.7	0	0
Church Creek (n = 29)	57.8 $\pm$ 9.4	11.9 $\pm$ 5.9	7.5 $\pm$ 4.3	6.0 $\pm$ 3.2	0

*bus purpureas* (lotus), *Dolichos biflorus* (horse gram), *Glycine max* (soybean), *Arachis hypogaea* (peanut), *Sambucus nigra* (elder), *Triticum vulgare* (wheat germ), and *Canavalia ensiformis* (Con A, type III, jackbean). The inhibition sugars used with each lectin are tabulated in Table 2. All lectins and inhibition sugars were purchased from Sigma Chemical Co. (St. Louis, MO).

Agglutination tests were carried out in 96-well U-bottom plates (Cell Wells, Corning, NY). Initial lectin-solutions were at a concentration of 0.1 mg/mL except that of Con A, which was at 1.0 mg/mL. All lectin solutions were prepared in phosphate-buffered saline (pH 7.4) and were serially diluted 2-fold with IS in microtiter plates to give final dilutions of 1:1 to 1:2048. To test sugar inhibition, each lectin was diluted serially in 0.2 M solutions of the appropriate inhibition sugar(s) (Table 2). Fifty  $\mu$ l of each hemocyte suspension was added to each experimental and control well, and the plates were incubated for 24 hours at room temperature (25°C).

All the hemocyte samples tested were from single oysters. The

number of oysters from which hemocytes were tested against each lectin is given in Table 1. The cells from an identical number of oysters from each collection site were employed in negative control tests (i.e., IS, instead of lectin) was used.

Preliminary and earlier studies (Cheng et al. 1980, 1993) had revealed that not all of the hemocytes exposed to the appropriate lectins agglutinated. Consequently, the percentages of clumped cells (i.e., number of clumped cells/100 cells) were ascertained at the highest concentration of each lectin tested as well as at dilutions of 1:64, 1:512, and 1:2048. The counting of agglutinated and single cells was achieved microscopically. When three or more cells were clumped, these were considered to be agglutinated. Pairs of cells were seldom observed.

Con A was included in every test as a positive control because it is known that it will agglutinate hemocytes of *C. virginica* (Yoshino et al. 1979, Cheng et al. 1980, 1993, Kanaley and Ford 1990).

TABLE 2.

Inhibition sugars tested against hemocytes from oysters from Malpeque Bay, Galveston Bay, and Church Creek in the presence of 9 lectins.

Lectin	Inhibition Sugar	Malpeque Bay	Galveston Bay	Church Creek
<i>Lathyrus odoratus</i>	N-acetyl-D-glucosamine	NI	NI	NI
	D(+)-glucose	NI	NI	NI
	D(+)-mannose	NI	NI	NI
<i>Limulus polyphemus</i>	N-acetylneuraminic acid	I(1:1)	I(1:2)	I(1:2)
	D-glucuronic acid	I(1:1)	I(1:64)	I(1:2)
<i>Tetragonolobus purpureas</i>	N-acetyl-D-glucosamine	I(1:64)	I(1:2)	I(1:2048)
<i>Dilochos biflorus</i>	N-acetyl-D-galactosamine	I(1:2048)	I(1:2)	I(1:1)
<i>Glycine max</i>	D(+)-galactose	I(1:2048)	I(1:512)	I(1:1)
<i>Arachis hypogaea</i>	D(+)-galactose	I(1:1)	I(1:1024)	I(1:1)
<i>Sambucus nigra</i>	N-acetyl-D-galactosamine	NI	I(1:2)	NI
	D(+)-galactose	I(1:1)	I(1:1)	NI
<i>Triticum vulgare</i>	N-acetylneuraminic acid	I(1:64)	I(1:1)	I(1:64)
	N-acetyl-D-glucosamine	I(1:1)	I(1:2)	I(1:2)
Con A	N-acetyl-D-glucosamine	I(1:64)	I(1:1)	I(1:512)
	D(+)-glucose	I(1:1024)	I(1:1)	I(1:2048)
	Sucrose	I(1:2048)	I(1:1)	I(1:1)

NI, no inhibition; I, inhibition of clumping (at the lowest effective lectin concentration).

### Statistical Analysis

To test for possible significance between the differences in the number of agglutinated hemocytes from Malpeque Bay or Galveston Bay oysters and those from Church Creek oysters that had been exposed to the *L. odoratus* lectin, the 2 sample t test (Neter et al. 1983) was employed.

## RESULTS

### Hemocyte Agglutination

The mean numbers of agglutinated hemocytes per 100 cells  $\pm$  SDs from oysters harvested from the 3 regions that had been exposed to 5 concentrations of each of the 9 lectins as well as the sample sizes are tabulated in Table 1.

### Inhibition Tests

The results of the inhibition tests involving saccharides known to inhibit cell agglutination mediated by each of the nine lectins tested with oyster hemocytes from the 3 regions are tabulated in Table 2. The lowest lectin titer at which each sugar was effective is also presented in Table 2.

### Agglutination Titers

The lowest agglutination titers of all of the 9 lectins reacted with hemocytes from oysters from the 3 locations are tabulated in Table 3.

## DISCUSSION

There were quantitative and qualitative differences in the reactions of the lectins tested against hemocytes from oysters collected from the 3 sites reported herein as well as between the present results and our earlier data (Cheng et al. 1993).

### Quantitative Differences

Quantitative differences are defined as differences in the lowest agglutination titers of different lectins when exposed to oyster

hemocytes. There were such differences associated with all of the 9 lectins reacted with hemocytes from oysters from the 3 locations (Table 3). For example, in the case of the *L. odoratus* lectin, the lowest agglutination titer for Malpeque Bay oyster hemocytes was 1:512 while it was 1:1024 for Galveston Bay and Church Creek hemocytes (Table 3). Similarly, the lowest titer for the agglutination of Malpeque Bay and Galveston Bay hemocytes by *L. polyphemus* lectin was 1:2048 and 1:1024 for Church Creek hemocytes (Table 3). Among the lectins tested, the most dramatic differences in the agglutination titers for oyster hemocytes from the 3 regions rest with the *S. nigra* lectin; the lowest titer for Malpeque Bay hemocytes was 1:512, for Galveston Bay hemocytes it was 1:64, and for Church Creek hemocytes it was 1:1024 (Table 3).

It is noted that at the highest concentration of the *L. odoratus* lectin tested (i.e., 1:1 dilution of 0.1 mg of lectin/mL), 61.50  $\pm$  4.82% of hemocytes from Malpeque Bay oysters and 68.12  $\pm$  0.59% of hemocytes from Galveston Bay oysters agglutinated (Table 1). *H. nelsoni* has not been reported from either of these regions (S. McGladdery, pers. comm., J. Couch, pers. comm.). On the other hand, only 43.77  $\pm$  2.41% of the hemocytes from

TABLE 3.

Lowest agglutination titers of the 9 lectins tested against hemocytes of *Crassostrea virginica* from 3 locations.

Lectin	Malpeque Bay	Galveston Bay	Church Creek
<i>Lathyrus odoratus</i>	1:512	1:1024	1:1024
<i>Limulus polyphemus</i>	1:2048	1:2048	1:1024
<i>Tetragonolobus purpureas</i>	1:1024	1:1024	1:2048
<i>Dolichos biflorus</i>	1:1024	1:512	1:2048
<i>Glycine max</i>	1:1024	1:64	1:1024
<i>Arachis hypogaea</i>	1:1024	1:512	1:1024
<i>Sambucus nigra</i>	1:512	1:64	1:1024
<i>Triticum vulgare</i>	1:1024	1:512	1:1024
Con A	1:512	1:512	1:1024

Church Creek, Johns Island, SC, were agglutinated by the *L. odoratus* lectin (Table 1). As stated earlier, a preliminary survey of Church Creek oysters conducted during February through March 1995, involving 50 specimens, revealed that approximately 4% harbored *H. nelsoni*. Although the percentage of agglutinated hemocytes from Church Creek oysters is significantly lower than those from oysters from the other 2 locations ( $P < 0.005$  in both comparisons), it is still relatively high when compared to the percentages of agglutinated cells from Chesapeake Bay oysters. Preliminary data indicate that in Chesapeake Bay, the clumping of hemocytes from native oysters when exposed to the *L. odoratus* lectin ranged from 2–16% (Cheng, unpubl.). The lower hemocyte agglutination percentage in the case of Church Creek oysters when compared to Malpeque Bay and Galveston Bay hemocytes is correlated with the presence of low prevalence (approximately 4%) of MSX.

That not all hemocytes were agglutinated by the lectins tested (Table 1) indicates that surfacial molecular heterogeneity exists among these cells. These differences may be correlated with the different populations, subpopulations, or ontogenetic stages of cells or with the physiologic state of the cells (see review, Cheng 1996).

Although there was some overlapping between the mean numbers of agglutinating hemocytes  $\pm$  SDs as the dilution of each lectin was increased, a trend is evident that as the concentration of each lectin was decreased, the number of clumped hemocytes/100 cells was also diminished (Table 1).

#### Qualitative Differences

Qualitative differences relative to the reactions of oyster hemocytes to lectins are defined as the nonagglutination of hemocytes from oysters harvested from one site and clumping of hemocytes from oysters from another when exposed to the same lectin. Also, the noninhibition of agglutination of hemocytes from oysters from one region and inhibition of agglutination of cells from oysters from another region by the same saccharide is considered to be a qualitative difference.

No qualitative differences were observed in the lectin-hemocyte combinations being reported in the present study; however, one such difference has been recognized between the results of this study and our earlier report (Cheng et al., 1993). Specifically, as indicated in Table 4, in our earlier study we reported that D-glucuronic acid did not inhibit agglutination of cells from Galveston Bay oysters exposed to the *L. polyphemus* lectin; however, in the present study it is being reported that this saccharidal residue inhibited clumping of hemocytes from oysters from the same area when exposed to this lectin, with the lowest effective lectin titer being 1:64. The remaining differences between the results obtained with Galveston Bay oyster hemocytes reported earlier (Cheng et al. 1993) and herein are minimal (Table 4). Nevertheless, these minimal differences may, in part, reflect seasonal changes as suggested by Kanaley and Ford (1990). This interpretation may be supported by the fact that the Galveston Bay oysters employed in the earlier study (Cheng et al. 1994) were collected during the last week in March through the second week in April 1992, while those employed in this study were harvested between the last week in January and the first week in March 1995, hence 1 to 3 months earlier in the year.

Also, a qualitative difference, as defined, existed between the inhibition of hemocyte agglutination exposed to the *S. nigra* lectin by N-acetyl-D-galactosamine. Specifically, this saccharidal resi-

TABLE 4.

Comparisons between the lowest concentrations of the saccharides that inhibited agglutination of hemocytes from Galveston Bay oysters exposed to nine lectins.

	Earlier Study (Cheng et al. 1993)	This Study
Con A		
N-acetyl-D-glucosamine	1:2	1:2
D(+)-glucose	conc.	1:2
Sucrose	conc.	1:2
<i>Tetragonolobus purpureus</i>		
N-acetyl-D-glucosamine	1:2	1:2
<i>Limulus polyphemus</i>		
N-acetylneuraminic acid	conc.	1:2
D-glucuronic acid	NI	1:64
<i>Dilichos biflorus</i>		
N-acetyl-D-galactosamine	conc.	1:2
<i>Glycine max</i>		
N-acetyl-D-galactosamine	1:32	—
D(+)-galactose	—	1:512
<i>Triticum vulgare</i>		
N-acetyl-D-galactosamine	conc.	1:2
N-acetylneuraminic acid	—	1:512
<i>Lathyrus odoratus</i>		
D(+)-glucose	NI	NI
D(+)-mannose	NI	NI
N-acetyl-D-galactosamine	—	NI
<i>Sambucus nigra</i>		
N-acetyl-D-galactosamine	—	1:2
D(+)-galactose	—	1:1
<i>Arachis hypogaea</i>		
D(+)-galactose	—	1:1024

—, not done; NI, no inhibition; conc., most concentrated solution tested, i.e., 0.2 M solution.

due did not inhibit the clumping of cells from oysters from Malpeque Bay and Church Creek; however, the agglutination of cells from Galveston Bay oysters was inhibited up to the 1:2 dilution (Table 2).

#### Nature of Lathyrose

Cheng et al. (1994) suggested that the presence of lathyrose on the surfaces of *C. virginica* hemocytes may serve as a marker for innate resistance to *H. nelsoni*. This hypothesis is based on the significantly higher percentage of lathyrose-positive hemocytes in oysters free of *H. nelsoni* and the extremely small number, if any, lathyrose-positive cells in *H. nelsoni*-infected oysters (Cheng et al. 1994, Cheng 1994, Cheng and Dougherty 1994). It remains to be determined, by challenge and/or sentinel studies, as to whether the presence of lathyrose is actually involved in resistance of *C. virginica* to *H. nelsoni*.

Although it is recognized that there have been transplantations of oysters along the Atlantic coast of North America and into and from the Gulf coast, there is evidence that oysters from the Atlantic coast and Gulf of Mexico have not interbred significantly. Specifically, Buroker (1983) has reported that there are differences in the frequencies of certain allozymes and proteins between Atlantic coast and Gulf of Mexico oysters. Also, Reeb and Avise (1990), by analyzing restriction site variation in mitochondrial

DNA, have concluded that oysters from the Atlantic coast are of a different population from those of the Gulf of Mexico and Karl and Avise (1992), by analyzing restriction fragment length polymorphisms in nuclear DNA, have arrived at the same conclusion. Thus, Atlantic and Gulf coast oysters comprise different evolutionary clades. Thus, differences between Malpeque Bay and Galveston Bay oysters (Tables 2, 3) may reflect cladal differences. However, differences between Malpeque Bay and Galveston Bay oysters and Church Creek oysters cannot reflect cladal differences. Based on currently available information, the significantly lower number of lathyrose-positive cells in Church Creek oysters can only be correlated with the presence of *H. nelsoni*.

As had been previously observed (Cheng et al. 1993, 1994), the agglutination of hemocytes exposed to the *L. odoratus* lectin is not inhibited by N-acetyl-D-glucosamine, D(+)-glucose, or D(+)-mannose, the usual inhibiting saccharides (Ticha et al. 1980) (Table 2). Specifically, Cheng et al. (1993) reported that hemocyte clumping resulting from exposure to the *L. odoratus* lectin is not inhibited by any of the concentrations of D(+)-glucose or D(+)-mannose employed. Subsequently, Cheng et al. (1994) reported that oysters collected from the South Carolina coast extending from Bull's Bay to Charleston Harbor and Apalachicola Bay, FL, included 70.47 and 70.13% of their hemocytes, respectively, that became clumped when exposed to the most concentrated solution of the *L. odoratus* lectin tested (i.e., 0.1 mg/mL). Furthermore, the percentages of clumped hemocytes decreased from 47.53 to 9.31% and from 42.55 to 19.19% in South Carolina and Apalachicola Bay oysters, respectively, when the lectin concentrations were reduced from 1:64 to 1:2048 from the highest concentration used. As indicated in Table 2, none of

hemocytes from oysters from all 3 regions included in this study were inhibited from clumping by N-acetyl-D-glucosamine, D(+)-glucose, or D(+)-mannose. Consequently the nature of "lathyrose" remains unknown.

#### Saccharidal Constituents

As a result of exposing hemocytes of *C. virginica* from the 3 regions to the 9 selected lectins and the testing of possible inhibition of cell agglutination by use of specific saccharidal residues, earlier reports (Kanaley and Ford 1990, Cheng et al. 1993) that 7 saccharides are associated with the hemocyte surfaces have been confirmed. Specifically, it has been confirmed that N-acetylneuraminic acid (which binds to *L. polyphemus* and *T. vulgaris* lectins), D-glucuronic acid (which binds to *L. polyphemus* lectin), N-acetyl-D-glucosamine (which binds to Con A, *T. purpureas*, and *T. vulgaris* lectins), N-acetyl-D-galactosamine (which binds to *D. biflorus* and *S. nigra* lectins), D(+)-galactose (which binds to *A. hypogaea* and *S. nigra* lectin), D(+)-glucose (which binds to Con A), and sucrose (which binds to Con A) are constituent saccharidal residues associated with the surface membranes of *C. virginica* hemocytes.

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## GAMETOGENESIS AND SPAWNING IN INTERTIDAL OYSTERS (*CRASSOSTREA VIRGINICA*) FROM WESTERN LONG ISLAND SOUND

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**ABSTRACT** Four intertidal populations of the eastern oyster, *Crassostrea virginica* were sampled monthly in western Long Island Sound from March 1993–February 1994. Patterns of gametogenesis and spawning were determined from histological preparations of gonadal tissue. At all sites 1 period of gametogenesis occurred, with rapid maturation of the gonad during May and June. Spawning was remarkably synchronous; all individuals collected showed evidence of gamete release. Most animals were spawning in August and early September, although limited spawning did occur in July. Temperature does not appear to be a critical factor for stimulating spawning in oysters from this part of the Sound. These findings were compared with those of Loosanoff and others for subtidal oyster beds from the same region.

**KEY WORDS:** Oyster, gametogenesis, spawning, intertidal, Long Island Sound

### INTRODUCTION

There is probably no other marine bivalve species inhabiting the east coast of North America that has received as much attention as the eastern oyster, *Crassostrea virginica*. A considerable amount of that information relates to aspects of the reproductive cycle of that species. Loosanoff (1942, 1965, 1966, 1969) was responsible for generating much of the literature for Long Island Sound oysters. His work on gametogenesis and spawning focused on subtidal oyster beds (mean low water to 40 feet in depth) located along the north shore of Long Island Sound, from New Haven Harbor to Bridgeport. The present study examines reproductive patterns of oysters from intertidal populations (above mean low water) in the same geographic area, in an attempt to determine whether or not maturation and spawning in intertidal populations of oysters occurs at the same time as it does in subtidal ones.

### MATERIALS AND METHODS

Oysters (*C. virginica*) were collected by hand from intertidal mudflats located at Milford Point, Milford, CT, Black Rock Harbor, Bridgeport, CT, Southport Beach, Fairfield, CT and the Saugatuck River, Westport CT (Fig. 1). Surface temperatures were taken almost daily at the Black Rock Harbor site during the time of high tide from May 1993 through February 1994. Monthly collections of oysters were made from March 1993 to February 1994 from all sites except Milford Pt. where sampling began in April 1993. Samples sizes varied from 9 to 21 oysters measuring 30.8 to 120.9 mm shell length from Bridgeport, 34.5 to 103.2 mm shell length from Milford, 25.1 to 112.3 mm shell length from Fairfield and 40.2 to 120.9 mm shell length from Westport. A total of 856 oysters were examined and used in the analysis of the reproductive cycle.

The oysters were measured along their greatest length ( $\pm 0.1$  mm), shucked and the visceral masses (gonad, liver and gastrointestinal tract) were removed and fixed in 10% buffered formalin. The tissues were then prepared for histological examination (Brousseau 1978). A microscopic examination was made of the visceral mass gonadal tissue before assigning each individual to one of five gametogenic categories according to the descriptions used by Brousseau (1987). Each oyster was assigned a gonadal index (GI) which corresponded to the 5 categories: indifferent/

inactive (0); spent (1); Spawning (2); early developing (3); late developing (4); and ripe (5). The mean monthly GI was calculated for each site and sampling period for both sexes combined.

The following categories of gonad development were used in this study. This protocol incorporated all of the stages used by Loosanoff (1942) in his study of oyster reproduction in Long Island Sound.

#### Categories of Gonad Condition

##### Indifferent/Inactive

Oysters with little or no follicular material present, making it difficult to determine sex in some cases.

##### Early Developing

Oysters characterized by the expansion of the follicle and the appearance of well-defined spermatogonia or oogonia along the follicle wall. A central lumen is present in each follicle.

##### Late Developing

Oysters in which the maturation of gametes is evident. Some ripe gametes appear in the central lumen.

##### Ripe

Female oysters with many mature, spherical oocytes, 45–50  $\mu$ m in diameter, that appear to be free within the follicular lumen. Male oysters having spermatids radiating toward the center of the follicle where they arrange themselves in radial columns.

##### Spawning

Oysters with reduced numbers of mature gametes in the follicles.

##### Spent

Oysters with ruptured follicles and residual gametes. Resorption of the gametes and reinvasion of the follicles by follicular cells ensues.

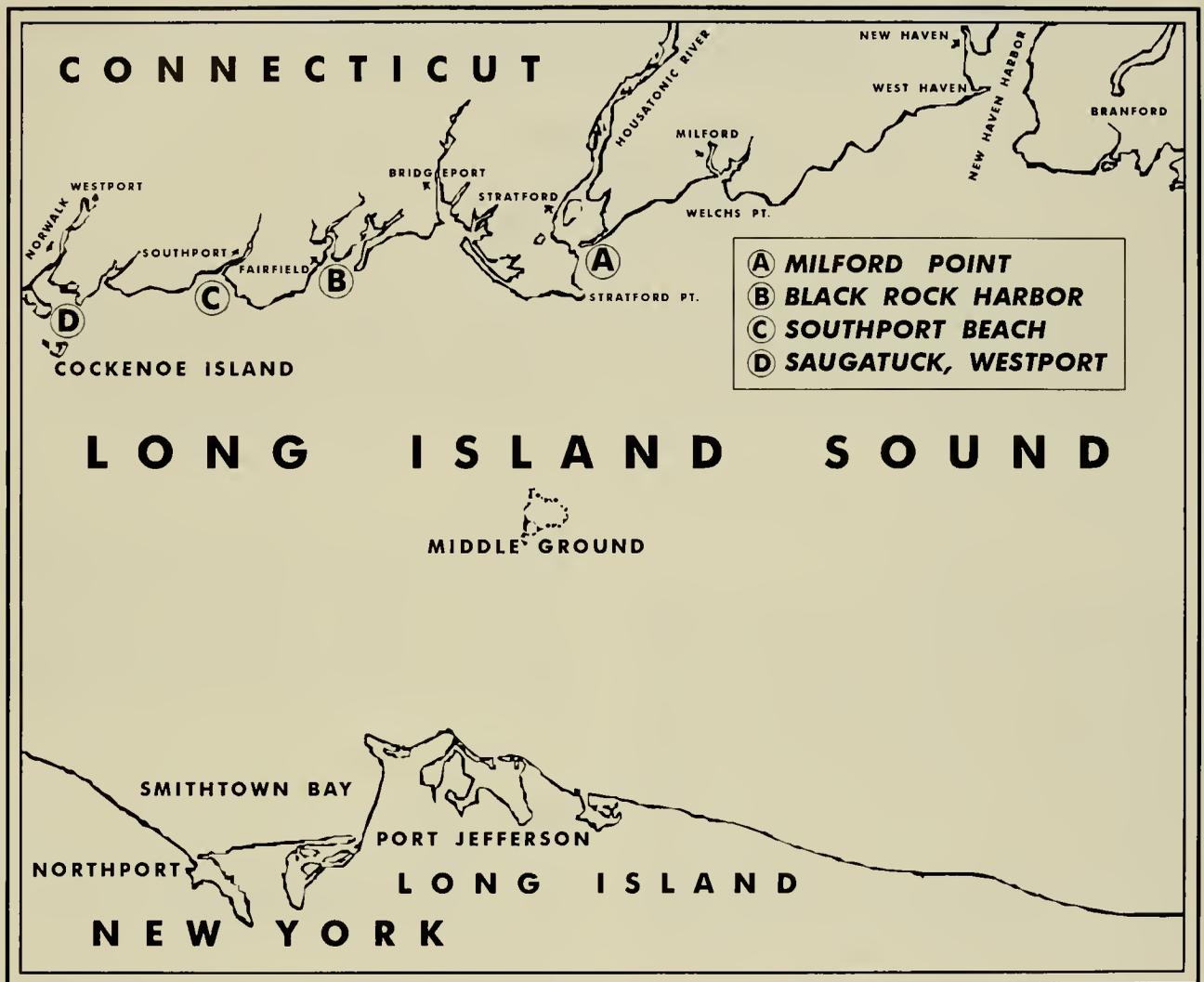


Figure 1. Map showing the locations of the 4 study sites: A) Milford Point, Milford, CT; B) Black Rock Harbor, Bridgeport, CT; C) Southport Beach, Fairfield, CT; D) Saugatuck River, Westport, CT.

## RESULTS

Reproductively active oysters were encountered from May through October at all sites except Southport Beach where active individual were collected from May through September (Fig. 2). At all sites, the pronounced winter dormancy period was followed by rapid maturation of the gonad during May and June, with fully ripe oysters (male and female) dominating the populations during July. The largest numbers of spawning oysters were found in August and September at each of the 4 sites, however, small numbers of oysters discharging gametes were encountered in July at the Milford Pt. and Southport Beach locations.

Monthly gonadal index values were remarkably consistent among the populations studied, especially at the Bridgeport, Milford and Westport sites (Fig. 3). Gonadal indices peaked in July in all populations (3.8–5.0) and fell precipitously in August (1.3–3.3), indicating a major spawn. The Southport population exhibited the least protracted spawning period, with 40% of the population releasing gametes in July. By October the GI had dropped to between 0.0 and 0.2 at all sites and did not increase until the following May with the onset of gametogenesis.

Spawning in the Bridgeport population was underway in August 1993 when surface water temperatures were close to the annual maximum (Fig. 4). Gametogenesis, on the other hand, occurred in the spring during a period of rapidly increasing water temperatures (May to June), when temperatures rose 5–8°C.

The sex ratios determined for oysters from the 4 populations are given in Table 1. Deviation from parity was observed at the Black Rock Harbor and Southport Beach sites, where the proportion of females in both populations was significantly higher than males. Incidence of hermaphroditism was low (<1%); hermaphrodites were reported from only two of the sites studied, Southport Beach and the Saugatuck River.

## DISCUSSION

This study of reproduction in 4 populations of *C. virginica* from Long Island Sound indicates that oysters living intertidally experience a single annual gametogenic and spawning cycle. Gametogenesis, which begins in May in these animals, is rapid and results in fully ripe individuals by June. Earlier studies (Loosanoff 1942, Loosanoff and Davis 1952) have shown that subtidal oyster

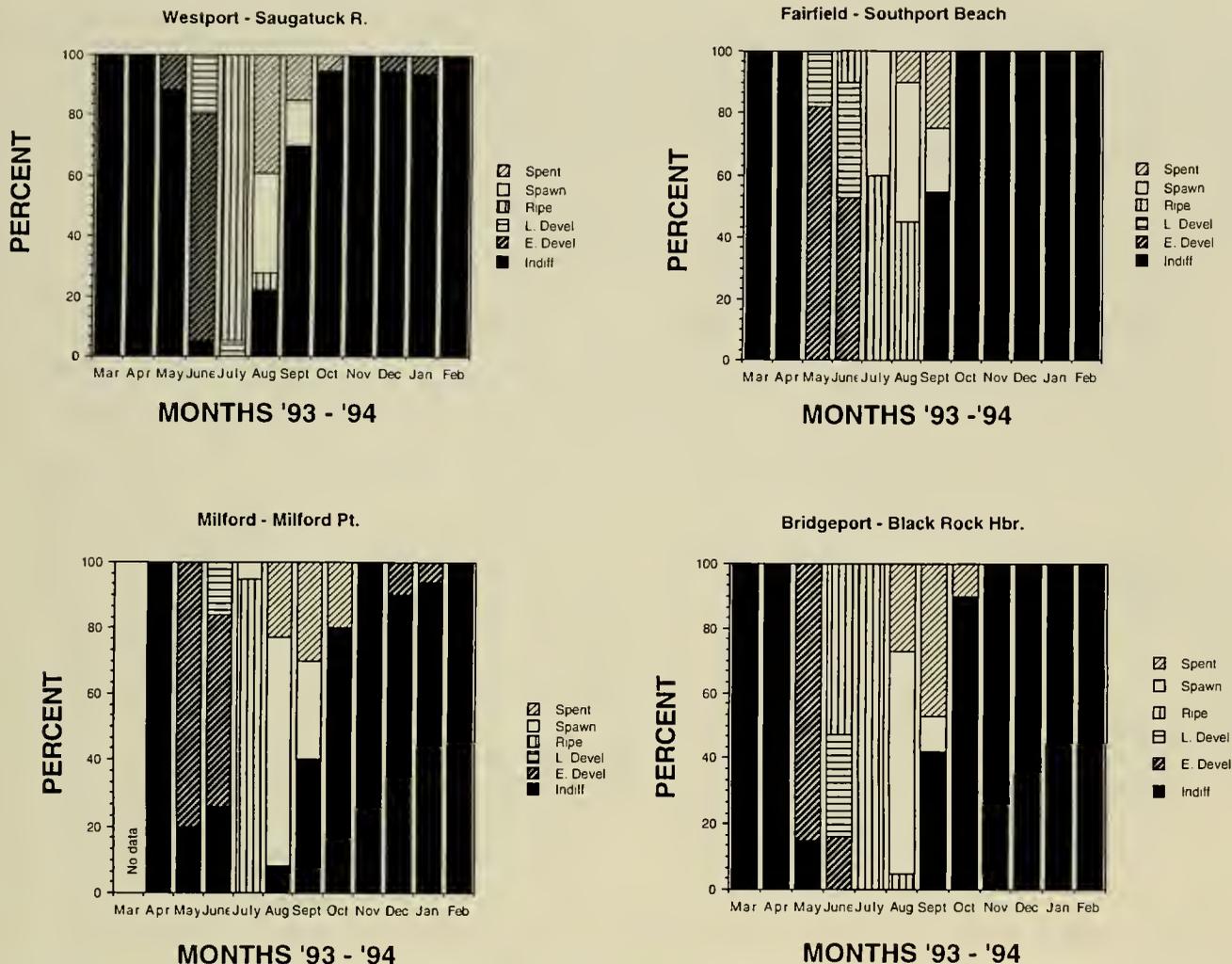


Figure 2. Percentage of *C. virginica* from Black Rock Harbor, Bridgeport, Milford Point, Milford, Southport Beach, Fairfield, and Saugatuck River, Westport with gonads in each developmental phase during 1993–1994.

populations from the same geographic locale experience 2 periods of gametogenic development, a major one in the spring coinciding with the one reported here for intertidal oysters and a second in the fall. Fall gametogenesis is arrested, however, in subtidal populations and does not resume until the following spring when conditions improve. The absence of a second gametogenic period in intertidal oysters may be due to a more rapid fall cooling of the shallow intertidal beds. The intertidal oysters showed very little variation in the degree of spring gonad development among individuals taken from the same area at the same time. This finding was consistent with that of Loosanoff (1942) for subtidal Long Island Sound oysters but varied from the observations made by Nelson (1928) on oysters from Barnegat Bay.

In general, the duration of spawning in *C. virginica* populations along the Atlantic coast varies geographically, increasing as latitude decreases. Oysters from Prince Edward Island, Canada spawn from late June to August (Kennedy and Battle 1964), a period similar in length and timing to that reported by Loosanoff (1942) for subtidal oysters in Long Island Sound. In Chesapeake Bay, there are reports of oysters spawning from June to September (Truitt 1929, Kennedy and Krantz 1982), whereas in populations

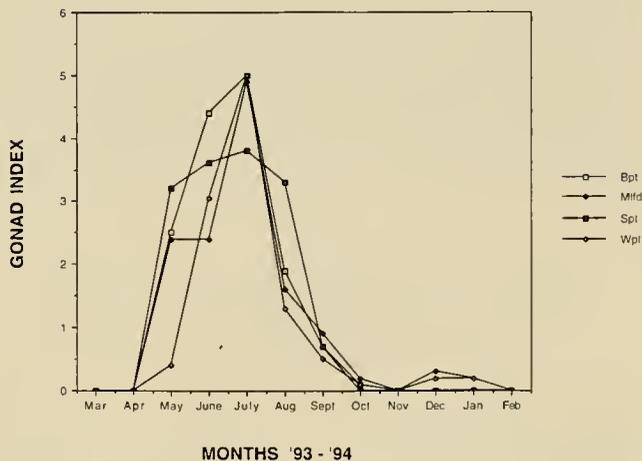


Figure 3. Mean monthly gonadal indices (GI) for *C. virginica* from the 4 populations sampled (Bpt = Bridgeport; Milfd = Milford; Spt = Southport; Wpt = Westport).

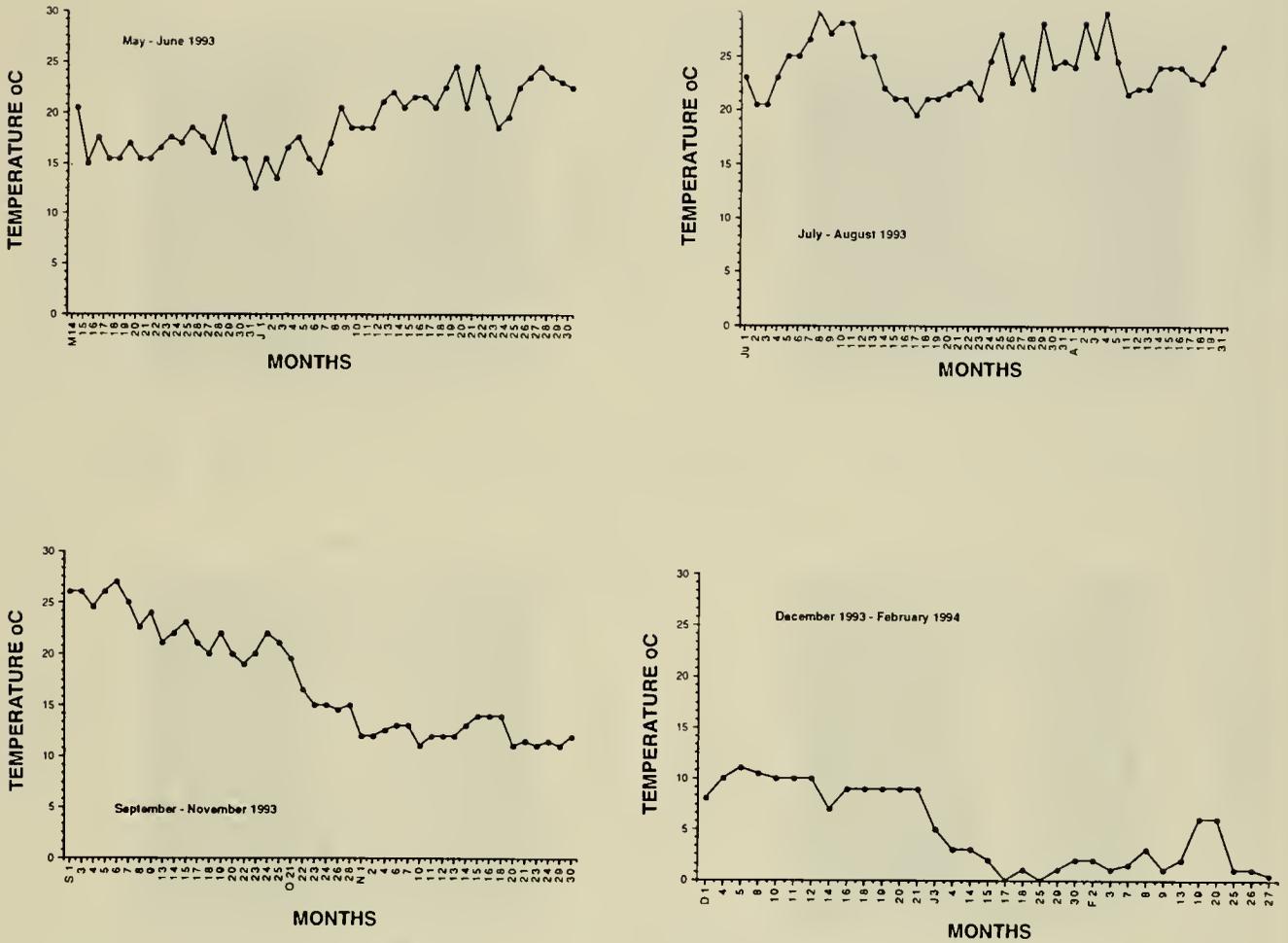


Figure 4. Temperature data for the Black Rock Harbor, Bridgeport sampling location during the period May 1993–February 1994.

from Wassaw Sound, GA, spawning has been observed from April through September (Heffernan et al. 1989).

No subtidal populations were included in this study, but the earlier work by Loosanoff and his colleagues (Loosanoff 1942, 1965, 1966, 1969, Loosanoff and Engle 1940, Loosanoff and Davis 1952) which covered almost 20 years, was so extensive that it provides a firm basis for comparison with the present study. In two of the intertidal populations studied here (Southport Beach and Milford Pt.) gamete release began in late June to early July. This timing coincides well with the onset of spawning reported for the

subtidal populations in the area. In the other 2 populations (Black Rock Harbor and Westport), however, spawning was delayed about 1 month. Spawning was not completed in all populations until the end of September, resulting in a slightly longer period for gamete release than seen in the subtidal populations. Within intertidal populations the spawning event was well synchronized; simultaneous spawning of males and females was observed in all populations.

Temperature requirements for gametogenesis and spawning in oysters have been the subject of much discussion, as they have for many other species of shallow-water marine bivalves. In Long Island Sound Loosanoff and Engle (1940) collected oysters with mature gametes from a subtidal population (30 ft depth) with maximum water temperatures less than 15°C. In intertidal populations, ripe individuals were not encountered until water temperatures had risen to 20°C indicating that in these populations gametogenesis is occurring under different temperature regimes. The role of temperature in triggering release of gametes seems even less clear. The temperatures at which spawning began in intertidal populations was above 20°C, almost 5°C higher than the required temperature for subtidal ones reported by Loosanoff and Davis (1952). It seems certain that factors other than temperature alone are important in triggering spawning in oysters and different triggering

TABLE 1.

Sex ratios of oysters from the 4 sampling locations in Long Island Sound.

Site	N	F:M
Milford Pt. Milford, CT	225	0.92:1
Black Rock Hbr. Bridgeport, CT	189	1.28:1*
Southport Beach Fairfield, CT	194	1.20:1*
Saugatuck R. Westport, CT	206	0.94:1

\* p < 0.05.

mechanisms may be operable in different parts of their geographic range (Kennedy and Krantz 1982).

Based on findings from previous studies (Coe 1932, Kennedy 1983), the sex ratios reported here were as expected. In two of the populations studied sex ratios were 1:1, whereas in the other two there was a preponderance of females. Since there is some indi-

cation that populations with older individuals have more females (Coe 1932), these differences in the sex ratios may have been due to different age structures. The low incidence of hermaphroditism in this study is similar to that noted in populations of this species from a number of geographic locations (Burkenroad 1931, Needler 1932, Coe 1934, Berg 1969, Kennedy 1983).

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## SUB-CLINICAL INFECTION OF OYSTERS (*CRASSOSTREA VIRGINICA*) (GMELIN 1791) FROM MAINE BY SPECIES OF THE GENUS *PERKINSUS* (APICOMPLEXA)

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**ABSTRACT** Using standard diagnostic techniques and a tissue culture medium previously described (Kleinschuster and Swink 1993), detection and select *in vitro* culture of species of *Perkinsus* isolated from Maine oysters were demonstrated. This is the first report of *Perkinsus* in oysters from Maine and the species is likely *P. marinus*.

**KEY WORDS:** *Perkinsus* sp., Maine, *Crassostrea virginica*, cell culture, sub-clinical infection

### INTRODUCTION

The protozoan *Perkinsus marinus* (Mackin, Owen and Collier 1950, Levine 1978) is a major parasite of the eastern oyster *Crassostrea virginica* (Gmelin 1791). Additionally, cross-transmission of other *Perkinsus* species among certain bivalve species has been demonstrated (Goggin et al. 1989). The geographic distribution of the *P. marinus* parasite has been well documented (Andrews and Hewatt 1957, Mackin 1962). Prior to this study, the northernmost occurrence of *P. marinus* or *Perkinsus* sp. infections in oysters appeared to be Cape Cod, MA (Susan E. Ford, pers. comm.). The objective of this research was to determine whether any *Perkinsus* species could be detected in clinically healthy oysters from Maine.

### MATERIALS AND METHODS

In early March 1995, clinically healthy oysters which had been spawned, cultured and grown-out to market size in the Damarascotta River in Maine were shipped to the Haskin Research Laboratory in Port Norris, NJ. Immediately upon arrival, the oysters were briefly washed with potable water to remove obvious debris. The oysters were notched and a 0.1 cc hemolymph sample was aspirated from the adductor muscle sinuses of each individual. A total of 70 hemolymph samples were screened for *Perkinsus*-like cells using phase microscopy. Whole oysters containing suspected parasites were diagnosed for *Perkinsus* sp. by traditional culture in Ray's fluid thioglycolate medium (FTM) (Ray 1954) followed by staining in Lugol's solution and/or by select *in vitro* culture of individual oyster hearts. Diagnoses of *in vitro* cultured parasites from oyster hearts as *Perkinsus* were via Ray's diagnostic method and the development of zoosporangia.

### RESULTS

Microscopic examination of hemolymph samples from 70 individual oysters revealed 18 oysters suspected to contain *Perkinsus*-like organisms, although intensity was light. These 18 whole oysters, including four with hearts removed for *in vitro* parasite propagation, following culture in FTM and staining with Lugol's solution, tested positive for the presence of a *Perkinsus* sp. in 14 of the 18 oysters (Fig. 1).

*In vitro* culture of hearts taken from 4 of the 18 evaluated

oysters, using previously described methods (Kleinschuster and Swink 1993), resulted in the establishment of four propagating *in vitro* cultures of *Perkinsus* (Fig. 2). Parasites from these cultures, following 4 days incubation in FTM, stained positively with Lugol's solution (Fig. 3) as described by Ray (1954) or formed zoosporangia with discharge tubes when incubated in sterile sea water, only following FTM treatment (Fig. 4).

### DISCUSSION

The finding of at least 1 species of *Perkinsus* in a substantial proportion (20%) of the oysters tested in this study indicates that *Perkinsus* occurs in Maine waters and is supported by the earlier discovery of *P. marinus* in *Macoma bathica* from Eastport, ME (Perkins 1993). Recognition that clinically healthy, commercially viable and marketable oysters may be parasitized with sub-clinical infections of this parasite is noted. Patent clinical manifestations and mortality of infected oysters in Maine are probably limited because of the prevalent colder waters to which the oysters are exposed as suggested by (Ragone-Calvo and Burreson 1994). However, as noted above, when isolated and cultured at higher temperatures *in vitro*, the parasites propagate readily. Sub-clinically infected oysters from cold waters introduced into warm waters may also develop clinical disease from parasite propagation; consequently, investigators may wish to intensify screening of experimental oysters prior to use.

Since selected *in vitro* cultured parasites in this study formed zoosporangia in sea water only after FTM incubation, the species most likely involved was *P. marinus* since *P. atlanticus* readily sporulates following transfer from the described culture medium directly into sea water (Kleinschuster et al. 1994).

### ACKNOWLEDGMENTS

This work is identified as Hatch Project No. D-32100-1-95 and identified as paper No. 95-8 by the Institute of Marine and Coastal Services, Rutgers University. We also wish to thank Dr. F. O. Perkins for helpful suggestions in the manuscript preparation.

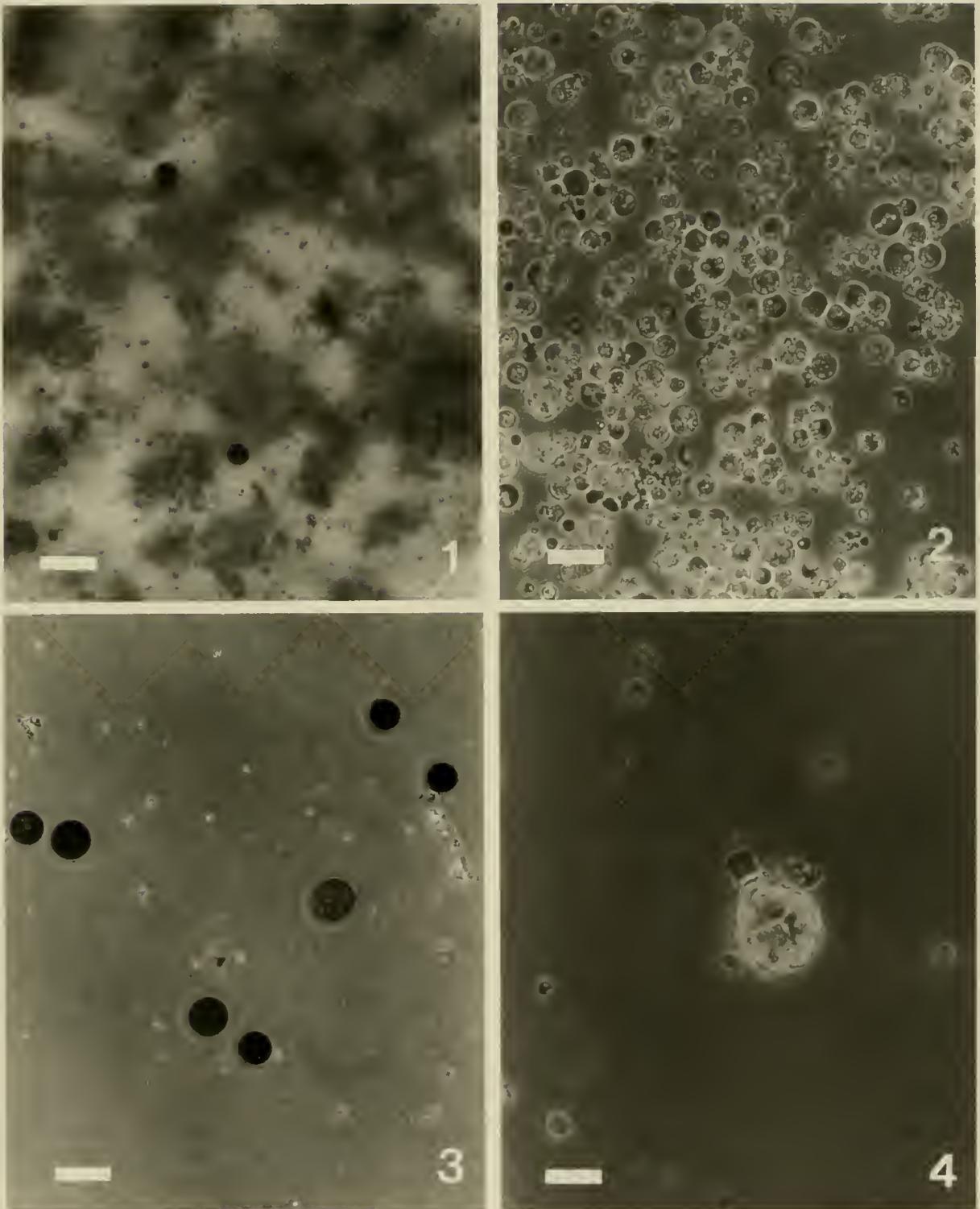


Figure 1. Photomicrograph of *Perkinsus* cells following culture in Ray's FTM and stained with Lugol's iodine. Squash preparation of whole body digestion. Scale bar, 0.1 mm.

Figure 2. Photomicrograph of early *in vitro* propagation of *Perkinsus* cells isolated from an oyster heart. Scale bar, 0.1 mm.

Figure 3. Photomicrographs of Lugol-stained *Perkinsus* cells following propagation in culture medium described, incubation in Ray's FTM and stained with Lugol's iodine. Scale bar, 0.1 mm.

Figure 4. Photomicrograph of a maturing *Perkinsus* zoosporangium revealing cellular division and a typical discharge tube. Developmental events followed successive culture in culture medium, Ray's FTM, and sterile sea water. Scale bar, 0.1 mm.

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## DETECTION OF *VIBRIO VULNIFICUS* AND *VIBRIO CHOLERA*E O1 IN OYSTER TISSUE USING IMMUNOELECTRON MICROSCOPY‡

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**ABSTRACT** Rabbit polyclonal antibodies (PAb) against purified cell wall capsular polysaccharide of clinical strains of *Vibrio vulnificus* and a monoclonal antibody (MAb) against the A antigen of the lipopolysaccharide (LPS) of *Vibrio cholerae* were evaluated for their usefulness in immunogold electron microscopy of *Vibrio* spp. in oyster tissues. The PAb to the *V. vulnificus* clinical strains did not label environmental isolates of this species, nor other *Vibrio* spp. Likewise MAb to *V. cholerae* O1 LPS did not cross react with other *Vibrio* spp., as tested by ELISA and immunogold electron microscopy. Both antibodies labeled pure cultures of the autologous strains and also bound to these phagocytized bacteria in oyster hemocytes. After 24 h exposure to *V. cholerae*, epithelia of mantle, gill, stomach, and muscle tissue showed low levels of labeling between cells, indicating that LPS material had been deposited there by phagocytic hemocytes involved in diapedesis. Whole cells of *V. cholerae* and *V. vulnificus* were only found in intact hemocytes and among stomach contents.

**KEY WORDS:** *Vibrio cholerae*, *Vibrio vulnificus*, oyster, immunogold, monoclonal antibody, electron microscopy, polyclonal antibody

### INTRODUCTION

Molluscan shellfish are an important vector of microorganisms which cause human illness (Food and Nutrition Board 1991). It is well documented that consuming raw shellfish can be hazardous to human health because their tissues concentrate substances in overlying seawater. Bacteria of human origin and indigenous species, such as *Vibrio cholerae* and *Vibrio vulnificus*, can cause severe human illness (Food and Nutrition Board 1991). However, for the latter species, predicting risk is especially difficult because the presence of vibrios has no relation to fecal coliform counts usually performed.

Depuration (controlled purification) is a post-harvest process which has been utilized for many years to reduce hazardous levels of bacteria in oysters. Low levels of *Escherichia coli* can be reduced in shellfish tissues in short time intervals (e.g., 48 hr); however, vibrio bacteria are retained for much longer (Jones et al. 1991, Tamplin and Capers 1992). Tamplin and Capers (1992) have shown that *V. vulnificus* densities in oysters cannot be reduced to low levels within 7 days, even in refrigerated seawater (e.g., 15°C), and that *V. vulnificus* replicates in oysters at higher temperatures. Likewise, Murphree and Tamplin (1995) reported that *V. cholerae* O1 is retained at high levels in oyster tissues after 48 hr treatment in controlled purification systems. These studies indicate that the persistence of vibrios in shellfish may be caused by bacterial adherence to oyster tissues and/or resistance to oyster host defenses.

Details of *in vivo* oyster-vibrio interactions have not been well defined, particularly because specific antibodies have not been available to discriminate a single species among the diverse bacterial flora of oysters. Harris-Young et al. (1993, 1995) have de-

scribed the effect of bacterial surface capsule on uptake and elimination of *V. vulnificus* by oyster phagocytic cells; however, their studies did not include immunomicroscopy techniques to locate specific species in hemocytes or other oyster tissues. Consequently, more specific tools, such as antibodies, are needed to further examine retention of vibrios in oyster tissues, especially species such as *V. cholerae* and *V. vulnificus* which cause human disease. These studies must examine whether oyster tissues can remove *Vibrio* spp. by phagocytic mechanisms previously described in earlier studies (Cheng 1975, 1977, Cheng and Rodrick 1975, Fisher 1988). A microscopic technique could accurately locate and identify pathogenic vibrio bacteria in oyster tissues, and determine which factors affect their persistence.

One promising approach to this problem is using immunolabeling techniques at the ultrastructural level (Roth 1983). Combining the relatively new gold-labeled antibody technique with specific antibodies to locate bacteria in ultrathin sections of oyster tissues should provide the high specificity and accuracy necessary to understand vibrio-oyster tissue interactions. Problems which must be addressed in order to validate this approach include successfully fixing and embedding oyster tissues in acrylic resins suitable for immunogold labeling, and obtaining antibodies specific for *V. vulnificus* and *V. cholerae* O1, but unreactive with other *Vibrio* spp. found in oysters. This paper reports the use of a specific polyclonal antibody (PAb) to capsular polysaccharide of a *V. vulnificus* clinical strain and a monoclonal antibody (MAb) specific to pathogenic *V. cholerae* O1 lipopolysaccharide, in immunoelectron microscopy studies to locate pathogenic vibrios in oyster hemocytes and other tissues.

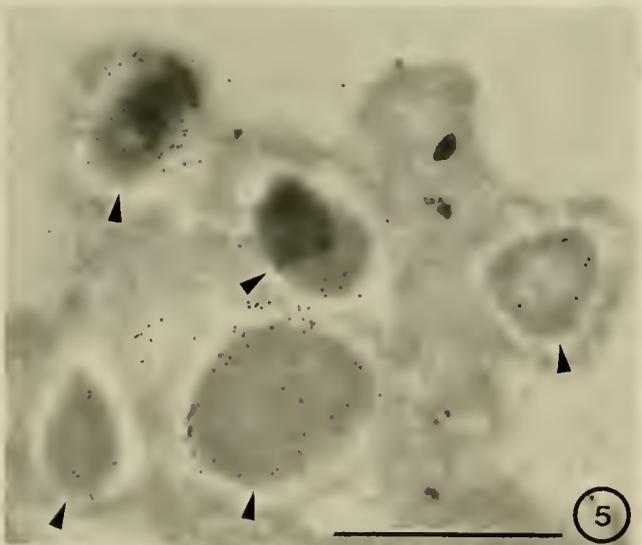
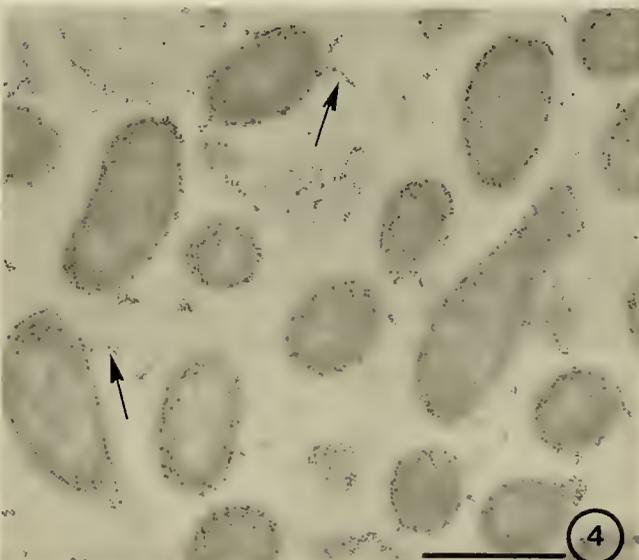
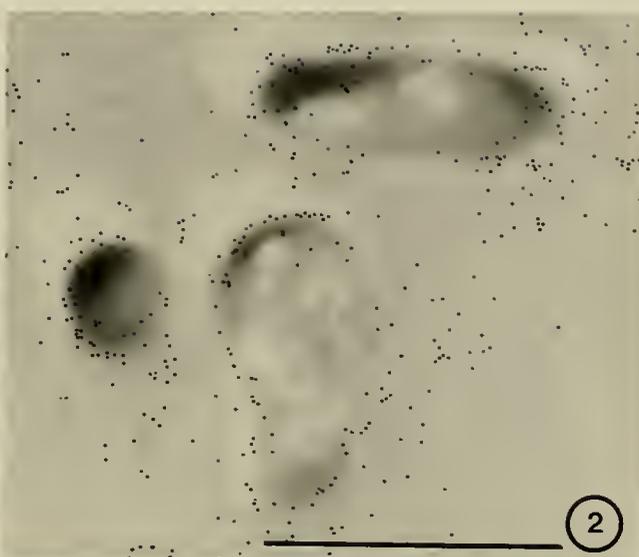
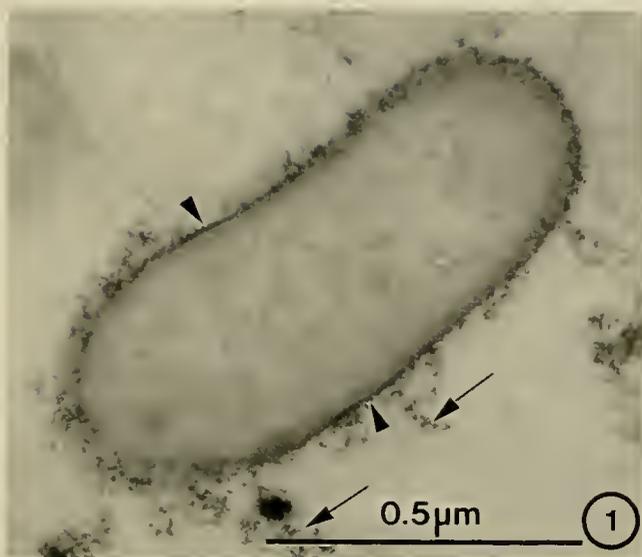
### MATERIALS AND METHODS

#### Antibodies

Rabbit PAb #1007 made against purified capsular polysaccharide of a *V. vulnificus* clinical strain was kindly supplied by Dr.

\*Corresponding author at Box 110700, University of Florida, Gainesville, FL 32611-0700. Phone (904)392-1096; Fax (904)392-5922.

‡Paper #R03093 from the Florida Agriculture Experiment Station.



R. J. Siebeling of Louisiana State University (Simonson and Siebeling 1993). Mouse MAb #4AG6 specific for the A antigen of the O1 lipopolysaccharide (LPS) of *V. cholerae* O1 was produced as mouse ascites (Brayton et al. 1987). Both antibodies have been extensively tested against numerous other bacterial genera and species to confirm specificity (Brayton et al. 1987, Simonson and Siebeling 1993).

#### Immunoelectron Microscopy

Cells of encapsulated *V. vulnificus* clinical strain #1002 (kindly provided by Dr. R. Siebeling of Louisiana State University) and *V. cholerae* O1 strain 124 (kindly provided by Dr. C. Carrillo, National Institutes of Health, Peru) were grown on tryptic soy agar (Difco Laboratories, Detroit, MI) for 24 hr at 35°C and then fixed for 20 min on ice in 1% glutaraldehyde/3% formaldehyde in sodium cacodylate buffer containing 0.6 M NaCl. Samples were dehydrated through a series of cold ethanol and embedded in Lowicryl HM-20 or LR White resins. Beginning with 75% ethanol, samples were processed at -20°C. Infiltration in the resin proceeded slowly, with 24 hr incubation in 30, 70 and 100% resins being required before UV polymerization of HM-20 at -20°C or chemical polymerization of LR White in an ice bath. Samples were routinely exposed to approximately 0.1 Torr vacuum for 5 min before polymerizing. Other Lowicryl resins (K4M and K11M) were also tried, and although they embedded bacteria and hemocytes, oyster tissue could not be successfully embedded. In order to observe the oyster tissue structures under more optimal preservation conditions, tissues were also fixed for 2 hr on ice in 2.5% glutaraldehyde/2.5% formaldehyde, followed by 2 hr in 1% osmium tetroxide. This material was embedded in Spurr's low viscosity epoxy resin.

For cytochemical visualization of surface polysaccharides in *V. cholerae* O1 and *V. vulnificus*, a protocol developed by Dykstra and Aldrich (1978) was employed. Bacteria were fixed in 2.5% glutaraldehyde containing 0.5% Alcian blue for 30 min on ice and then postfixed for 1 hr on ice in 1% OsO<sub>4</sub> containing 0.05% ruthenium red. Subsequent steps resembled those described above for morphological preservation.

For immunogold staining, the usual procedure was to cut ultrathin sections, collect them on Formvar-coated nickel grids, and block non-specific binding sites by floating for 5 min on 0.5% nonfat dry milk. All solutions were made or diluted with 0.5 M NaCl, 0.6% TRIS buffer (pH 7.2), 0.1% Tween 20. Primary antibody incubation proceeded for 1 to 18 hr at 4°C, followed by 1 hr on gold-labeled (10 nm) goat anti-mouse or anti-rabbit secondary antibody. Controls included normal mouse or rabbit serum and secondary antibody alone. The antibodies also were tested for labeling ability against several other clinical and numerous envi-

ronmental *V. vulnificus* isolates. Sections were usually post-stained very briefly (1 min or less) in uranyl acetate and lead citrate before viewing. All microscopy was performed with a Zeiss EM-10 CA transmission electron microscope.

#### *Vibrio*-Oyster Tissue Interactions

The localization of *V. vulnificus* and *V. cholerae* O1 in oyster tissues following laboratory exposure was studied. Oyster tissues for study were taken from *Crassostrea virginica* freshly collected in July 1992 at Cedar Key, FL, or at Apalachicola, FL in August 1993, and shipped to Gainesville, FL by overnight mail at 10°C. Oysters were placed in a tank containing *V. cholerae* O1 or *V. vulnificus* for time periods ranging from 2 to 24 hr. Following exposure, bacterial counts, determined by the Most Probable Number method, were determined to be approximately 10<sup>4</sup> for each *Vibrio* spp./g oyster meat. At each time interval, tissues fixed included hemocyte cells from adductor muscle sinuses and pieces of mantle, gill, muscle, stomach and intestine.

In separate experiments, *Vibrio* spp. were added to monolayers of hemocytes as single and mixed cultures to study microscopic events related to uptake and survival. Hemolymph was withdrawn from the adductor muscle and spread on tissue culture dishes (Harris-Young et al. 1993). After allowing 30 min for attachment, hemocytes were covered with a single bacterial species or a mixture (2 × 10<sup>7</sup> cells/mL) for 30 min. Plates were then washed gently and covered with sterile sea water for the balance of the experiment, until fixed.

In some experiments, hemocytes were exposed to both *V. cholerae* O1 and *V. vulnificus* simultaneously, and then fixed for study. Thin sections of these cells were first labeled with *V. vulnificus*-specific rabbit antiserum, followed by goat anti-rabbit antibody carrying a 10 nm gold label. They were then exposed to mouse MAb to *V. cholerae* O1, followed by goat anti-mouse labeled with 18 nm gold. The method thus differentiated *V. vulnificus* from *V. cholerae* cells by different sizes of gold particles. This allowed studies of preferential uptake of species, and whether a single hemocyte could phagocytize and digest both *Vibrio* spp.

## RESULTS

After Alcian blue-ruthenium red stain of polysaccharides, *V. cholerae* O1 and *V. vulnificus* cells were stained in a continuous surface layer corresponding to the LPS layer of the wall, and in capsular material extending from the cell surface outward (Fig. 1 illustrates *V. cholerae*). Mouse ascites fluid containing *V. cholerae* O1-specific MAb against A antigen of LPS effectively labeled thin sections of pure cultures of this bacterium at dilutions of 1:10,000. Figure 2 shows a continuous label along the cell surface

**Figure 1.** Cell of *V. cholerae* O1 stained with Alcian blue-ruthenium red protocol to demonstrate polysaccharides. Continuous wall layer of LPS has stained (arrowheads), as has capsular material extending out from cell surface (arrows). ×80,000.

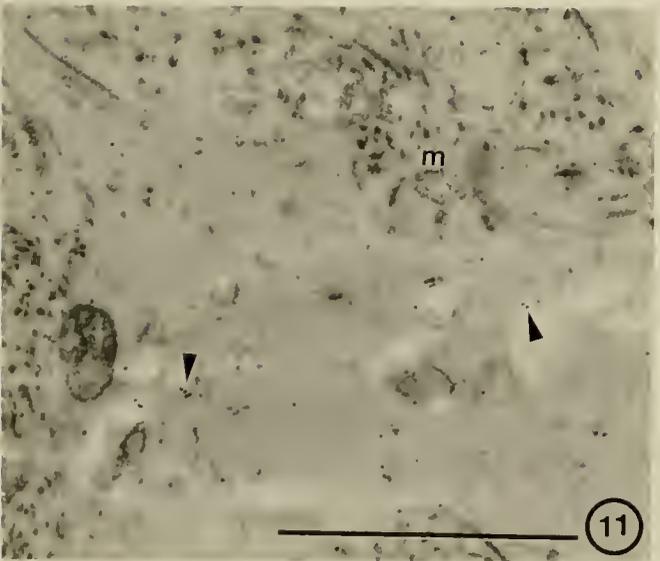
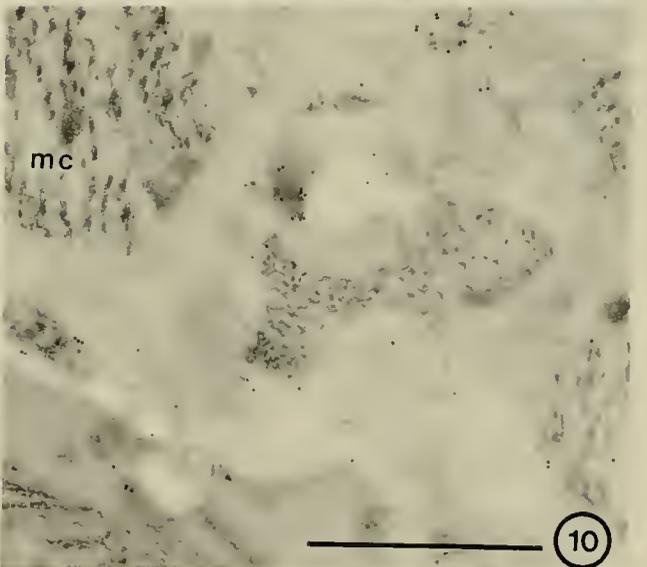
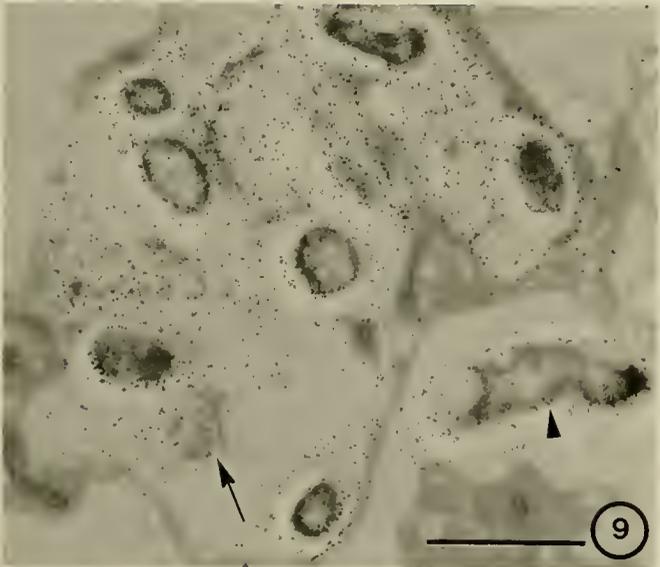
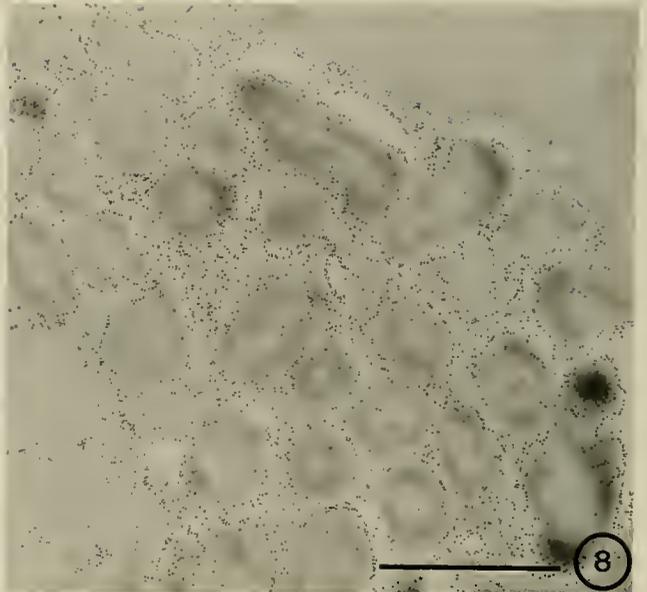
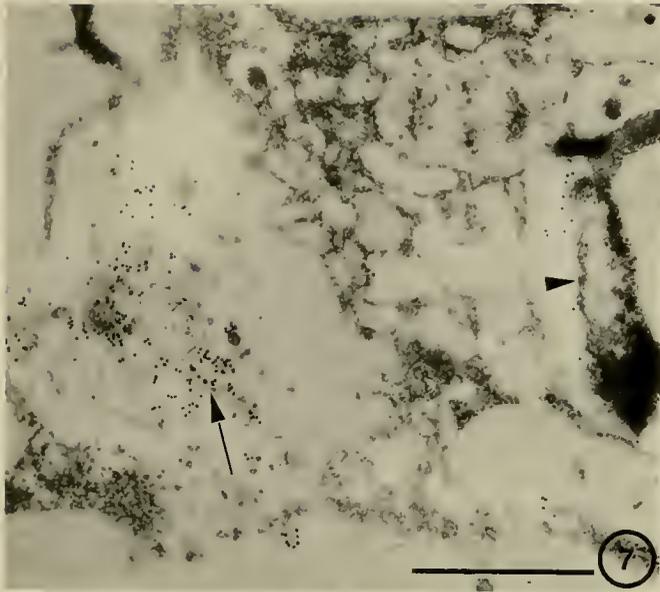
**Figure 2.** Pure culture of *V. cholerae* O1 strain 124 labeled with MAb to *V. cholerae* O1 LPS, 1:10,000 dilution. Label is seen over the cell walls. Label outside cells has bound to sloughed LPS surrounding the walls. Empty plastic did not label. ×40,000. Marker represents 1 μ.

**Figure 3.** *V. vulnificus* strain 4965 treated with *V. cholerae* O1-specific MAb as in Figure 2. No gold particles are visible. ×32,000. Marker represents 1 μ.

**Figure 4.** Pure culture of *V. vulnificus* strain #1002 labeled with polyclonal antibody #1007. Gold label indicating antibody binding is present over the periphery of the bacteria in capsular regions. Some capsular strands extend outward (arrows). ×19,000. Marker represents 1 μ.

**Figure 5.** Oyster phagocytic hemocyte containing numerous *V. vulnificus* cells 1 hr after exposure. Several intact bacteria (arrowheads) inside cytoplasmic lysosomes exhibit gold label. ×31,000. Marker represents 1 μ.

**Figure 6.** Hemocyte 2 hr after exposure to *V. vulnificus*. As in Figure 5, several labeled, intact bacteria are visible. ×31,000. Marker represents 1 μ.



plus some adjacent extracellular material sloughing from the cell surfaces. There was no labeling of a control sample of *V. vulnificus* (Fig. 3). Secondary antibody and normal serum controls were also negative (not shown).

Figure 4 illustrates a similar trial with *V. vulnificus* PAB #1007. Polyclonal antibody was applied to a thin section of a pure culture of *V. vulnificus* #1002. The entire cell surface labeled, as did a few loose strands of capsule extending outward from the cells. The labeled region corresponded in appearance to a continuous thin layer of capsule plus some free strands. More extra-wall material would have been labeled except that it often sloughed off during dehydration and embedding unless the Alcian blue-ruthenium red fixation was used. Only two (i.e., #1002 and #1005) out of the five tested clinical *V. vulnificus* isolates labeled with PAB. Pure cultures of *V. cholerae* O1 did not label (data not shown), nor did environmental isolates of *V. vulnificus*.

Hemocytes were exposed to the *Vibrio* spp. for periods ranging from 2 to 24 hr, and then fixed, embedded, sectioned, and labeled. After exposure to bacteria, hemocytes were not washed assiduously, and undoubtedly still continued to phagocytize bacteria for several hours, since many free bacteria remained attached to the culture dish (not shown). Figure 5 illustrates a hemocyte 1 hr after exposure to *V. vulnificus*. Several intact, labeled bacteria were present in lysosomes in the hemocyte cytoplasm. Similar images were common at 2 hr (Fig. 6). By 24 hr, however, (Fig. 7) lysosomes often showed diffuse label associated with fibrous contents; sometimes no bacteria were evident. Results with *V. cholerae* O1 were similar (Figs. 8 and 9). In Figure 8, contrast is low because poststain was eliminated to emphasize the amount of gold label present. The entire cytoplasm of this phagocytic cell was packed with lysosomes filled with labeled *V. cholerae* O1 cells. Figure 9 shows a hemocyte 16 hr after exposure to *V. cholerae*. Again, many labeled bacteria fill the lysosomes, and one partially digested bacterium is visible, indicating ongoing phagocytosis and digestion. A labeled bacterium can also be seen outside the phagocyte. Similar images of actively digesting phagocytes were obtained up to 24 hr after bacterial exposure. From about 3 hr onward, fragmented bacteria of both species and empty, labeled lysosomes began to appear, representing partially digested bacteria and undigested LPS and/or capsule.

In muscle, mantle, and gill tissue of oysters exposed to *V. cholerae* for 24 hr, hemocytes sometimes were seen containing phagocytic vesicles. Between the cells of mantle and muscle tissue, diffuse labeling could be seen, suggesting recent discharge of undigested LPS material from residual bodies of phagocytic hemo-

cytes (Figs. 10 and 11). Whole bacteria were not observed between cells of mantle, stomach, gills, or muscles, nor were they seen inside epithelial or other cell types in these tissues. Bacteria were observed mixed with ingested material within the oyster stomach, where they were evidently engulfed by wandering phagocytic hemocytes.

In double labeling experiments, some phagocytic hemocytes contained both *V. vulnificus* and *V. cholerae*, (Fig. 12), where both 10 nm gold (*V. vulnificus*) and 18 nm gold (*V. cholerae*) labels could be seen.

## DISCUSSION

Several embedding resins worked successfully for immunogold labeling of pure cultures of *V. vulnificus* and *V. cholerae* O1. However, only Lowicryl HM-20 and LR White proved equal to the problems of embedding oyster tissue for thin sectioning and immunogold labeling. Vacuum infiltration of the final plastic mixture was essential. Polyclonal antibody #1007 labeled capsules of *V. vulnificus* clinical isolates #1002 and #1005 and was useful on ultrathin sections of oyster tissues. It did not label other clinical isolates nor any environmental isolate tested. This high specificity did not adversely affect the studies. Instead, it provided greater assurance that the cells stained by antibody were the cells we added to oyster and hemocyte preparations. Monoclonal antibody #4AG6 to *V. cholerae* O1 LPS labeled ultrathin sections of all O1 strains extremely well. Both antibodies lacked cross-reactivity with other *Vibrio* spp. and with oyster tissues.

*V. vulnificus* has been reported to exist as opaque and translucent colonial morphotypes, with the difference being the quantity of polysaccharide capsule expressed on the cell surface (Simpson et al. 1987). We believe that a possible small population of translucent cells in our seed preparations did not affect interpretation of the studies as described above. First, the transition from the opaque to translucent form is at a very low rate, such that translucent cells, if present, would not have been observed at high sample magnifications. Second, even translucent forms of *V. vulnificus* produce capsule, and allow the antibody to label the cell surface. Differences in uptake and survival of translucent versus opaque *V. vulnificus* strains in oyster hemocytes have been previously reported by Harris-Young et al. (1993, 1995).

Our observations on oyster tissue suggest that bacteria are removed from the environment, in part by phagocytosis, primarily by phagocytic cells in the hemolymph. The fragmented appearance of some of the bacteria within phagosomes (Fig. 9) suggests

**Figure 7.** Hemocyte as in Figure 6, but 24 hr after exposure to bacteria. One labeled intact bacterium is visible (arrowhead), while in another lysosome a large area of fibrous material has labeled (arrow). This represents undigested capsular polysaccharide.  $\times 24,000$ . Marker represents 1  $\mu$ .

**Figure 8.** Hemocyte exposed to *V. cholerae* O1 cells for 16 hr, then fixed, and labeled with MAB. All bacteria are intact and heavily labeled. This section was not poststained, to emphasize the amount of gold present.  $\times 23,000$ . Marker represents 1  $\mu$ .

**Figure 9.** Hemocyte treated as in Figure 8. One labeled bacterium (arrowhead) is seen outside the cell. Several labeled bacteria and extensive free label representing undigested LPS are present in lysosomes. One partially digested bacterium is present (arrow).  $\times 21,000$ . Marker represents 1  $\mu$ .

**Figure 10.** Mantle tissue from oyster exposed to *V. cholerae* for 24 hr, showing diffuse label in regions between cells. No label occurs over muscle cells (mc) or other adjacent cells.  $\times 31,000$ . Marker represents 1  $\mu$ .

**Figure 11.** Adductor muscle (m) tissue as in Figure 10, showing diffuse label (arrowheads) between cells. No label is visible over cytoplasm of cells. This diffuse label is believed to be indigestible LPS from the *V. cholerae* wall exocytosed from phagocytic hemocytes.  $\times 40,000$ . Marker represents 1  $\mu$ .

**Figure 12.** Hemocyte from double label experiment. Two bacteria labeled with 18 nm gold particles are *V. cholerae* O1 (arrows), and a single *V. vulnificus* cell is seen at lower right as determined by its smaller 10 nm gold label (arrowheads).  $\times 44,000$ . Marker represents 1  $\mu$ .

that the oyster hemocytes can successfully degrade ingested cells of both *Vibrio* spp. by lysosomal processes. Our observations support the scheme proposed by Fisher (1986), suggesting that phagocytosis of bacteria occurs by hemocytes in the stomach, digestion in the lysosomal compartment, and discharge of residual material and nutrients in peripheral areas of the mantle, gills, and muscle. In a later review, Fisher (1988) referred to this process as diapedesis. By using antibodies to demonstrate the existence of LPS antigens in intercellular spaces of mantle, muscle, and gills of oysters exposed to *V. cholerae* for 24 hr, we have provided evidence for this process that ordinary morphological observations cannot. The possibility that the low level of label in the intercellular spaces was due to non-specific deposits during immunogold processing was not likely, since our staining and washing techniques yielded very clean control preparations with little or no background binding (Fig. 3).

In a previous study (Tamplin and Capers 1992), it was shown that all oyster tissues tested can harbor *V. vulnificus*, with 55 and 35% of total vibrios being found in digestive tract and adductor muscle respectively in untreated oysters. In the laboratory, these counts declined with time, indicating some removal of bacteria by oyster tissues. These data are consistent with our observations. Most of the vibrios we have seen in oyster tissues examined with the electron microscope have been free forms in contents of the digestive tract and in hemocytes withdrawn from adductor muscle for phagocytosis experiments. Tamplin and Capers (1992) simply performed microbiological counts; their methods would not have distinguished between viable bacteria in phagocytic hemocytes in adductor muscle and free bacteria within the muscle tissue. We have not observed free bacteria in adductor muscle; we found them only in hemocyte lysosomes.

There is other recent evidence of removal of vibrios from environmental waters by oyster hemocytes *in vitro*. Harris-Young et al. (1995) showed hemocytes capable of reducing vibrio counts by phagocytic activity. Opaque strains of *V. vulnificus* were more resistant to phagocytosis and degradation than translucent strains, but both were degraded to some extent.

It is also clear that there is no phagocyte specificity for *V. cholerae* O1 or *V. vulnificus* separately, since the single phagocyte in Figure 12 contained cells of both *Vibrio* spp. The tissues shown in Figures 10 and 11 had been exposed to *V. cholerae*. The stray diffuse labeling in these 2 figures is likely due to undigested LPS expelled from depleted lysosomes of phagocytes. In a recent study

of phagocytosis of *V. cholerae* O1 cells by rat intestinal epithelium (Sincharoenkul et al. 1993), specialized cells in Peyer's patches phagocytized and digested bacteria. Despite extensive searches for phagocytic activity in epithelia of all oyster tissues we examined, including intestinal regions, we failed to find any intact bacteria in lysosomes of epithelial cells, suggesting that phagocytic hemocytes are the primary sites of bacterial entrapment in the oyster.

In general our ultrastructural observations of hemocytes agree with the findings of Auffret (1988); we observe the same cell morphology and inclusions. We likewise conclude that a comprehensive study of oyster hemocyte morphology and function is indispensable for accurate classification of cell types. In an earlier study, Cheng (1977) presented ultrastructural evidence that bacteria are catabolized to glycogen in the lysosomal compartment of hemocytes, and that the glycogen is released into the hemocyte cytoplasm by lysosome dissolution or into the oyster tissues by exocytosis. We saw only a few glycogen particles in secondary lysosomes and no large cytoplasmic aggregates of glycogen rosettes such as Cheng (1975) described. This discrepancy might be explained by the oysters being from different sources or being collected at different times of the year. Fisher (1988) has shown that rates of hemocyte migration and phagocytosis are dependent on ambient temperatures and that hemocyte activities in general are depressed by high water temperatures. The fact that our oysters were fixed during July and August may explain the absence of large glycogen deposits as seen in Cheng's (1975) material.

This paper shows for the first time the feasibility of locating and labeling cells of human pathogenic bacteria inside cells of the oyster, and opens the way to understanding how oyster tissues and hemocytes interact with bacteria in their environment. The study represents the first results of planned experiments of oyster hemocyte structure and function.

#### ACKNOWLEDGMENTS

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## REVIEW OF ECOLOGY AND FISHERY OF THE OLYMPIA OYSTER, *OSTREA LURIDA* WITH ANNOTATED BIBLIOGRAPHY<sup>1</sup>

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**ABSTRACT** The Olympia oyster, *Ostrea lurida*, is a small bivalve mollusk species native to the western United States and Canada. It was commercially important in the late 19th century, and was cultured in Washington State until a near-collapse of the industry in the 1950s. Since then it has made a minor commercial comeback, but has been largely superseded by the introduced *Crassostrea gigas*. Most significant literature dates from prior to the collapse of the industry in the 1950s, and much of this is comprised of state or federal agency reports, or similar obscure literature formats. This document is divided into 2 parts; a review of all important literature to date on the distribution, biology, ecology, taxonomy, and commercial exploitation of *O. lurida*; and an annotated bibliography of known literature pertaining to *O. lurida*.

### CLASSIFICATION

Phylum MOLLUSCA  
Class BIVALVIA  
Subclass PTERIOMORPHIA  
Order OSTREOIDA  
Superfamily OSTREACEA  
Family OSTREIDAE  
*Ostrea lurida* Carpenter, 1864  
*Ostrea conchaphila* Carpenter, 1857, possible synonym  
Preferred common name: Olympia oyster  
Other common names: native oyster, California oyster, Yaquina oyster

### IDENTIFICATION AND MORPHOLOGY

*Ostrea lurida* is a small oyster. Specimens in Bamfield Inlet on Vancouver Island may exceed 6 cm in shell height; those currently found in Humboldt Bay, CA, and the southwest Puget Sound, WA, rarely exceed 5 cm (Baker, unpubl. data); those in San Francisco Bay are usually much smaller (Townsend 1893, Edmondson 1923). The shells are roughly elliptical or circular in outline, and the longest axis is normally from the hinge to the ventral margin (Figs. 1 and 2). The lower, or attached valve (left valve) is not deeply cupped, in contrast to the genus *Crassostrea*. The internal volume of a market-sized *O. lurida* from the southwest Puget Sound is about 4 cubic cm (Westley 1961).

The shell of *O. lurida* is thin and not chalky, as are members of the genus *Crassostrea*. There is usually no external sculpture other than an irregular series of concentric lines and often a plicate ventral (opposite hinge) margin, but Stafford (1915) reported a small population of oysters on Campbell Island, British Columbia, with deep, narrow shells and frequently with radiating ridges, which he positively identified as *O. lurida*, and the *O. lurida* illustrated by McLachlan and Ayres (1979) have radial ridges. External shell color is usually gray, but may be blotched with purple (Carpenter 1864a, Hertlein 1959). Specimens from Humboldt Bay, CA, are usually brown (pers. obs.); a yellow, striped form identified by Carpenter as *expansa* can be found in southern California; a purple form identified by Carpenter as *lauticauda* is found in San Pedro Bay, CA (Hertlein 1959), and a

white variety was reported by Stafford (1915) in Oyster Lagoon, in Blunden Harbor, British Columbia. According to Morris (1966), *lauticauda* is relatively elongate, while the rounder *expansa* is more likely to have a fluted or zigzag margin. There is no noticeable periostracum in mature specimens, but juveniles may have a thin yellowish periostracum (Baker, unpubl. data).

The interior of the shell is coated with a thin nacreous-like layer that quickly wears off in dead specimens (pers. obs.). This layer is greenish throughout most of the range (Kozloff 1973, 1974), but Stafford (1915) reports it as white in most of British Columbia. A form from San Quentin Bay, CA, and identified by Carpenter as *rufoides*, has reddish blotches on the interior of the shell, especially in the vicinity of the muscle scar (Hertlein 1959). The muscle scar is normally unpigmented or very slightly pigmented (Kozloff 1974). The shell is made of 2 main calcitic layers; an outer simple prismatic layer, and an inner foliated layer, in which the calcite crystals are organized in spirals that form stepped-growth pyramids (Watabe 1988).

On the inner margin of the shells on either side of, but not a part of, the hinge are a series of 2-12 tiny pits in the lower, or attached valve, and corresponding denticles on the upper valve. They are termed "chomata", with the pits being "catachomata" and the denticles being "anachomata" (Torigoe 1981). Some authors use the less precise term "crenulate" for these denticles (e.g., Quayle 1960) (Figs. 1 and 2).

The soft tissue is distinguished from sympatric (introduced) species in the genus *Crassostrea* by a lack of a promyal chamber, or an exhalant opening dorsal to the adductor muscle. Elsey (1935) includes good illustrations of the comparative anatomy of *O. lurida* and *Crassostrea gigas*. The gill ostia of *O. lurida* are 90-180  $\mu\text{m}$  long and 45-60  $\mu\text{m}$  wide, or about one-third larger than those of *C. gigas* (Elsey 1935). The diploid chromosome number is 20 (Ahmed and Sparks 1967). Cytological and other differences are discussed by Ahmed (1975).

### FOSSIL RECORD

Arnold (1909) states that *O. lurida* is present in late Miocene deposits in central California, and Howard (1935) reports this species in Pliocene deposits. All other records are more recent. *O. lurida* is a fairly abundant fossil along most of its present range (Dall 1897, Arnold 1903, Filice 1958, Valentine 1959, 1960, Kvenvolden et al. 1979, Atwater et al. 1981, Clifton 1983, Miller and Morrison 1988), and is sometimes the dominant fossil.

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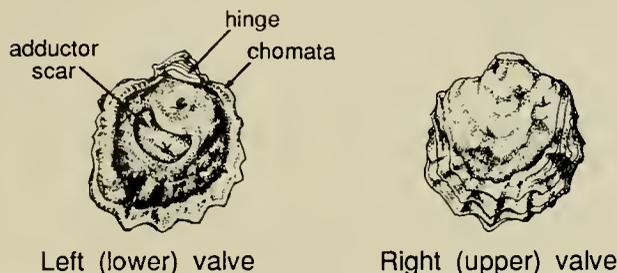


Figure 1. *O. lurida* shells from Vancouver Island, British Columbia, shown life size.

DISTRIBUTION

Although *O. lurida* is capable of living in full seawater, its distribution throughout the majority of its range is restricted to isolated bays and estuaries. The latitudinal extremes of the distribution are unclear. Dall (1914) states that the northernmost limit of *O. lurida* is Sitka, AK, and the southernmost limit is Cabo San Lucas, Baja California Sur (Mexico), but does not cite a reference for either locality. Paul and Feder (1976) state that this species is present in southeast Alaska, but do not name any localities.

In British Columbia, Stafford (1915) found *O. lurida* in a large number of inlets from Campbell Island south, and suspected that they were in many more. Quayle (1969) states that *O. lurida* is present in every small, shallow inlet in British Columbia. Large (commercially harvestable) populations of *O. lurida* on the inland waters, from north to south, have been reported in Hecate Channel by Campbell Island, Fish Egg Inlet, Blunden Harbor, Hardy Bay, Quadra Island, Malaspina Inlet, Oyster River, Comox Harbor, Pender Harbor, Deep Bay, Nanoose Bay, Oyster Harbor, Boundary Bay, and Ladysmith Harbor, Saltspring Island, and Portage Inlet, in Victoria (reported variously by Stafford 1915, Elsey 1933, and Quayle 1969). *O. lurida* larvae have been reported in abundance in Pendrell Sound and Hotham Sound (Bourne and Heritage 1979). From north to south on the outer coast of Vancouver Island, large populations of *O. lurida* have been reported in Quatano Sound, Esperanza Inlet, Nootka Sound, Toquart Harbor, Barkley Sound, and Sooke Harbor (Elsy 1933).

In Washington, *O. lurida* is found throughout the inland waters and in Willapa Bay (Galtsoff 1929) and possibly Grays Harbor (D.

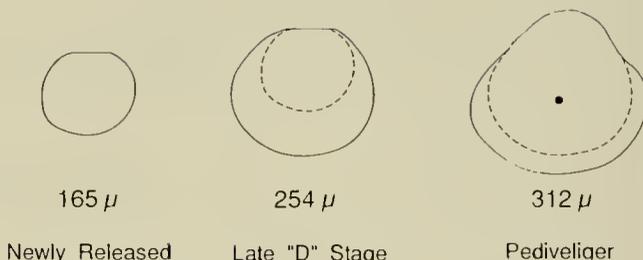


Figure 3. Outlines of *O. lurida* veliger larvae at 3 stages of growth.

Tufts, Washington Dept. Fish. shellfish biologist, pers. comm.), along the outer coast. There are no records of *O. lurida* in Grays Harbor in the literature, however, so it must be regarded as uncommon there, at best. Past or present commercial quantities have been reported in Bellingham Bay and Samish Bay in northern Washington, Discovery Bay on the northeast corner of the Olympic Peninsula (Townsend 1893), Hoods Canal, all the inlets of the southwest Puget Sound, and Willapa Bay (Galtsoff 1929).

In Oregon, *O. lurida* has been reported in Netarts Bay, (Edmondson 1923, Dimick et al. 1941, Marriage 1954), Yaquina Bay (Fasten 1931, Dimick et al. 1941), and Coos Bay as an introduced species (Edmondson 1923, Carlton 1988, Carlton, pers. comm.), although *O. lurida* was abundant in Coos Bay within the last few thousand years (Dall 1897). Commercial quantities were found in Netarts Bay (Edmondson 1923) and Yaquina Bay (Fasten 1931, Dimick et al. 1941). *O. lurida* has apparently died out of Netarts Bay (L. Hansen, Whiskey Creek Oyster Farms, Netarts, OR, pers. comm.). It was introduced to Alsea Bay (Mix and Sprague 1970) and Winchester Bay (R. Sardiña, Winchester Aquaculture, Winchester Bay, OR, pers. comm.), with unknown results. There are no reports of *O. lurida* in any other Oregon estuaries.

In California, Galtsoff (1929) reported *O. lurida* as common on protected rocky reefs, but Bonnot (1935) did a survey of all California estuaries and bays, and found *O. lurida* absent in a number of bays north of Point Conception. *O. lurida* was present at that time in Humbolt Bay, Tomales Bay, San Francisco Bay, Elkhorn Slough in Monterrey Bay, and all bays and estuaries that Bonnot investigated south of Point Conception, including Mugu Lagoon, Alamitos Bay in San Pedro Bay, Anaheim Bay, Newport Bay, Mission Bay, San Diego Bay, and Tijuana Lagoon. *O. lurida* is reported as a major component of an Indian shell midden at Patrick's Point (Barner 1981), probably too far north of Humbolt Bay to have been carried from there, which may indicate the past abundance of *O. lurida* at Big Lagoon, just to the north. C. Johnson (Johnson Oyster Co., Inverness, CA, pers. comm.) reports that *O. lurida* are common but not abundant in Drakes Estero. Gilbert (1891) reported them in Bolsa Bay in Huntington Beach (which is now continuous with Anaheim Bay), and Coe (1932b) and Coe and Allen (1937) found them consistently at the Scripps Pier in Morro Bay. Turner et al. (1965) reported *O. lurida* in an intertidal survey off San Elijo Lagoon in San Diego County, but since they reported the size of "up to five inches," much larger than the normal size of *O. lurida*, this record is in doubt. On the other hand, because *O. lurida* has appeared in some southern California lagoons and estuaries after they were dredged to permit regular seawater exchange (see below), it is possible that there are marine populations nearby which supply recruits to coastal harbors.

Commercial quantities of *O. lurida* have existed in Humbolt

ARCATA BAY, HUMBOLT BAY, CA

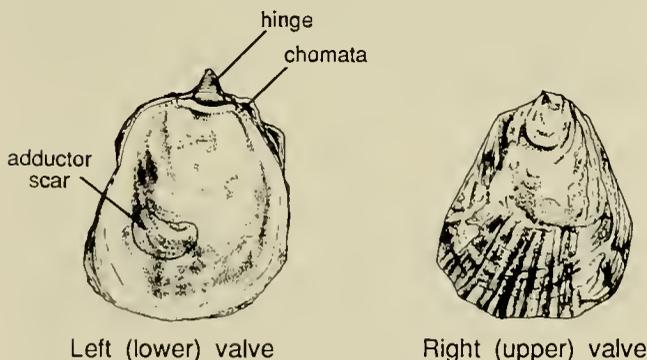


Figure 2. *O. lurida* shells from Humbolt Bay, CA, shown life size.

Bay (Bonnot 1936), Tomales Bay (Barrett 1963), southern San Francisco Bay (Packard 1918), and Elkhorn Slough (Smith and MacKenzie 1948).

More recent surveys of many southern California lagoons and estuaries do not report *O. lurida* at some locations where they were formerly reported. This is the case with Elkhorn Slough (Browning 1972), Mugu Lagoon (Peterson 1975, Onuf 1987), Alamitos Bay (Reish 1963), Anaheim Bay (Reish et al. 1975), Mission Bay (Fry and Croker 1934), San Diego Bay (Browning and Speth 1973), and Tijuana Lagoon (Peterson 1975). Since the dredging and placement of riprap at the mouths of Agua Hedionda (Bradshaw et al. 1976) and Los Penasquitos Lagoons (Mudie et al. 1974), however, *O. lurida* has become established in both lagoons, where historically it was absent.

In Mexico, *O. lurida* has been reported in Bahia de San Quintin, in Baja California Norte (Keen 1962, Barnard 1970). The only other mention the author was able to find was the often-cited but unexplained mention of Cabo San Lucas in Baja California Sur by Dall (1914), which is at the extreme southern tip of the Baja peninsula. Keen (1958), however, reported no *O. lurida* on the Baja peninsula from Cedros Island south, but instead reported the morphologically very similar, but virtually unstudied, *Ostrea conchaphila* Carpenter, 1957. *O. conchaphila* is about the same size as *O. lurida* and differs from southern California varieties of *O. lurida* only in subtle differences that grade into *O. lurida* (Hertlein 1959). All Mexican records of *O. lurida* and *O. conchaphila* should probably be reviewed in this light.

*O. lurida* has been introduced to Japan (Hori 1933, Imai et al. 1954), and apparently it is established there, at least as a cultured species, because an introduction of *O. lurida* in 1970 to South Korea was made from Japan (Bae and Bae 1972). An attempt early in this century to introduce *O. lurida* to Hawaii failed (Brock 1960).

The larvae of *O. lurida* apparently tend to stay near their place of origin, since they are rarely, if ever, reported in near shore coastal plankton. This, plus the evidence that although *O. lurida* died out in prehistoric times in Coos Bay, OR (Dall 1897), and has only recently reappeared there (Carlton 1988), probably by human aid (L. Qualman, Qualman Oyster Farms, Charleston, OR, pers. comm), suggest that the rate of genetic exchange between coastal populations in Washington, Oregon, and northern California, is low. Table 1 shows distances between known coastal populations of *O. lurida* from Grays Harbor, WA, to the California-Mexico border, at about 1900, and Table 2 shows the same for after 1970.

#### HABITAT

*O. lurida* is moderately euryhaline, with about 80% survival at 15 ppt salinity for 49 days (Gibson 1974), although the lower limit of large populations in the southwest Puget Sound is about 23–24 ppt, average, for winter months (Hopkins 1937). Quayle (1941) states that populations in British Columbia are found near the mouths of small rivers in inlets. Coe (1932) and Coe and Allen (1937) reported them as a common fouling organism in full seawater in La Jolla Bay in California.

The northern limit is apparently set by temperature; *O. lurida* cannot withstand freezing (Davis 1955), and needs water of at least 12.5°C to reproduce (Hopkins 1937). Stafford (1915) was of the opinion that the northernmost populations of *O. lurida* persist only because they exist in sheltered inlets that warmed in the summer. It is not clear what the upper thermal limit is, but adults can withstand 30°C for a few hours (Hopkins 1937).

TABLE 1.

Distances between known coastal bays and estuaries with *Ostrea lurida*; known distribution about 1900.

Bay or Estuary (North to South)	Distance to Next	Reference
Grays Harbor, WA	32 km	Galtsoff 1929
Willapa Bay, WA	172 km	Galtsoff 1923
Netarts Bay, OR	91 km	Edmondson 1923
Yaquina Bay, OR	431 km	Fasten 1931
Humbolt Bay, CA	325 km	Bonnot 1935
Tomales Bay, CA	30 km	Bonnot 1935
Drakes Estero, CA	50 km	C. Johnson, pers. comm.
San Francisco Bay, CA	136 km	Packard 1918
Elkhorn Slough, CA	460 km	Bonnot 1935
Mugu Lagoon, CA	91 km	Bonnot 1935
Alamitos Bay, CA	3 km	Bonnot 1935
Anaheim Bay, CA	5 km	Bonnot 1935
Bolsa Bay, CA	26 km	Gilbert 1891
Newport Bay, CA	102 km	Bonnot 1935
La Jolla Bay, CA	12 km	Coe 1932b
Mission Bay, CA	14 km	Bonnot 1935
San Diego Bay, CA	18 km	Bonnot 1935
Tijuana Lagoon	—	Bonnot 1935

*O. lurida* is only rarely reported in benthic invertebrate surveys of water more than a few meters deep, although it is present in the main shipping channel, at a mean depth of over 10 m, in Isthmus Slough of Coos Bay, OR (Baker, unpubl. data), and the main beds in Yaquina Bay, OR were also in the main shipping channel (Fasten 1931, Dimick et al. 1941). Hopkins (1937) reported that this species prefers shallow subtidal areas or large tide pools, but individuals can be found at least 2 m above mean low water in the intertidal as well (Baker, unpubl. data).

*O. lurida* apparently requires hard substrate to settle on, but readily settles on very small pieces of hard substrate (Fasten 1931). This allows the species to form loose reefs in soft mud areas, and the largest populations occur in low intertidal or shallow subtidal mud areas of estuaries (Townsend 1893, Stafford 1915, Galtsoff 1929, Quayle 1941, 1960). Fairly large populations can also occur on rocky reefs (Stafford 1915, Bonnot 1935), and individuals or clusters are common on rocks in parts of the Puget

TABLE 2.

Distances between coastal bays and estuaries with *Ostrea lurida*; 1970 and later.

Bay or Estuary (North to South)	Distance to Next	Reference
Grays Harbor, WA	32 km	D. Tufts, pers. comm.
Willapa Bay, WA	152 km	D. Tufts, pers. comm.
Yaquina Bay, OR	263 km	Wachsmuth, 1979
Coos Bay, OR	120 km	Carlton, 1988
Humbolt Bay, CA	325 km	J. Carlton, pers. comm.
Tomales Bay, CA	30 km	C. Johnson, pers. comm.
Drakes Estero, CA	50 km	C. Johnson, pers. comm.
San Francisco Bay, CA	136 km	Bradford & Luoma, 1980
Newport Bay, CA	74 km	Human, 1970
Agua Hedionda Lagoon, CA	24 km	Bradshaw et al., 1976
Los Penasquitos Lagoon, CA	—	Mudie et al., 1974

Sound, WA (pers. obs.), in inlets of British Columbia (Quayle 1969), and in California (Galtsoff 1929, MacGinitie 1935, Barnard et al. 1959, Kozloff 1973). Kozloff (1973) also states that in parts of San Francisco Bay, *O. lurida* is a common fouling organism on pilings and floating piers.

### LIFE HISTORY

*O. lurida* is a protandrous hermaphrodite. The gonads form at about 8 weeks after settlement, the spermatogonia are mature at five months, and the oogonia are mature at age six months. The sexual cycle after the initial male phase follows a female-male-recuperation stage cycle (Coe 1931a, 1932a, 1934). "Sperm balls" may function as spermatozeugmata when filtered from the water by a female (Strathmann 1987). The length of the male-female cycle varies with the individual, and may be interrupted at any stage by low temperature and carried on again when the temperature increases again (Coe 1931a). Hundreds of thousands of sperm balls may be released, each with about 2000 sperm (Coe 1931b), and the average number of larvae released during the female stage is about 250,000 (Hopkins 1936), but the number of eggs prior to fertilization has not been reported. Although the sexual stages may overlap, self-fertilization generally does not occur (Coe 1931b).

Spawning begins in California when the water temperature reaches 16°C in southern California, and lasts at least 7 months (Coe 1931a); in the southwest Puget Sound when the water temperature reaches 12.5–13°C, and lasts for about 6 months (Hopkins 1937, Santos et al. 1992b); and in Japan when the water temperature reaches 14°C (Imai et al. 1954). These temperatures are average daily temperatures taken in the main channel, and in the shallows water temperature may easily exceed that (pers. obs.). Santos et al. (1992b) reported that gametogenesis and spawning occurred at temperatures between 12 and 21°C in the laboratory, but while specimens at 12°C took 8 weeks from the beginning of gametogenesis to spawning, those at 21°C took only 2–3.5 weeks. Females at 18 and 21°C in this study produced significantly more larvae than those at lower temperatures. In British Columbia the spawning season lasts about 3 months (Stafford 1915). Two spawning/swarming peaks per year are common, if not typical (Hopkins 1937).

Eggs have been reported to be 90 µm (Elsey 1935) to 100–110 µm (Loosanoff and Davis 1963) in diameter at maturity. The larvae are brooded by the female about 10–12 days (Coe 1931a, Hopkins 1937, Strathmann 1987), and are gradually released in a process termed "swarming" by Stafford (1915), at about 180–185 µm in diameter (Stafford 1915, Hori 1933, Loosanoff and Davis 1963). Information on growth is included in Loosanoff and Davis (1963), and figures of larvae in various stages of growth are included in Hori (1933) and Loosanoff et al. (1966). The larvae spend from 21 days (pers. obs.) to 1 month (Hopkins 1937) to 6 to 8 weeks (Breese 1953) in the plankton, and settle at about 300 µm in diameter (Hori 1933, Hopkins 1937, Loosanoff and Davis 1963). They seem to prefer the undersides of objects (Hopkins 1935, 1937), although Bonnot (1937b) found that they would settle on upper or lower surfaces of concrete slats stacked several high with a narrow space in between, where it was dark. Although *O. lurida* larvae settle readily on concrete (Bonnot 1937b, Hopkins 1937), they will apparently not settle heavily on brush, as do larvae of *Crassostrea* species (Stevens 1928). Two setting peaks per year are typical (Bonnot 1937b, Hopkins 1937), but recruit-

ment intensity is not necessarily in direct proportion to the magnitude of the spawning peaks (Hopkins 1937). The percent survival from recruitment to age 6 months for juveniles (spat) in southwest Puget Sound in the early 1950s averaged about 3%, with a 3% standard deviation (calculated from Woelke 1958).

*O. lurida* nears maximum size in about 4 years, growing relatively little after that (D. McMillin, Olympia Oyster Co, Shelton, WA, pers. comm.), but the growth rate of juveniles has not been studied. The present author has found fossil shells of individuals in Coos Bay, OR, with ten or more major hinge annuli, which may correspond to age in years.

### PATHOLOGY AND POLLUTION

*O. lurida* is relatively disease-free, compared to other oyster species, although several virus-like lesions and several proliferative diseases have been reported at a low incidence (Mix 1976a, 1976b, Mix and Riley 1977). A haplosporidium (Mix and Sprague 1970) and a possibly pathogenic flagellate of the genus *Hexamita* (Stein and Denison 1959) have also been reported. None of these were correlated with significant mortalities in *O. lurida*, but Elston (1990) has incorrectly stated that *Hexamita* has been established as a disease-causing agent. A "microcell" disease described as *Mikrocytos mackini* that caused significant mortalities in *O. edulis* and *C. gigas* in British Columbia was discovered intermittently in *O. lurida* from Yaquina Bay, OR, in the winters of 1969 and 1970, but no mortality studies were done on it in *O. lurida* (Farley et al. 1988). Korringa (1976) makes reference to an unknown disease (not *Hexamita*) that occasionally destroys significant numbers of *O. lurida* in Washington.

Cardwell et al. (1979) suspected that blooms of the dinoflagellates *Gymnodinium splendens* and *Ceratium fusum* cause *O. lurida* mortalities, but D. McMillin (pers. comm.) reported good *O. lurida* survival, compared to *C. gigas*, during blooms of these dinoflagellates, which he had positively identified from photomicrographs taken during the bloom.

The only common internal macroparasite is an intestinal copepod, *Mytilicola orientalis* Mori, 1935, formerly called *M. ostreae*, introduced from Japan (Wilson 1938, Bernard 1969). The incidence of infection is low, ranging from 0 to 3% in San Francisco Bay, with no apparent effects (Bradley and Seibert 1978), and from 0 to 16% in the southwest Puget Sound, with a corresponding slight decrease in body weight (Odlaug 1946). *Odostomia* spp. (Gastropoda), which are ectoparasites, have been reported in high numbers in association with *O. lurida* (Strong 1928), but there is no evidence that *Odostomia* has a significant effect on the population.

The most serious form of pollution for *O. lurida* has been waste sulfite liquor from pulp mills. Studies in the southwest Puget Sound indicated high mortality at high concentrations of sulfites, and a lowering of body weight and reproductive success at lower quantities (Hopkins et al. 1935, McKernan et al. 1949, Odlaug 1949, Stein et al. 1959a, Gunter and McKee 1960). Oyster culturists in the area noted a total loss of *O. lurida* beds in inlets with pulp mills (Oakland Bay and Budd Inlet), and a general decline in the rest of the region (D. McMillin, pers. comm.).

Galtsoff (1929) suggested that untreated sewage dumped directly into Budd Inlet, in south Puget Sound, had killed the *O. lurida* there. Beck et al. (1966) review bacterial depuration in *O. lurida*. Clark et al. (1974) and Gibson (1974) found only minor effects of marine fuel contamination on *O. lurida*. Modin (1969),

reported high concentrations of organo-chloride pesticide residues washed into some California estuaries and taken up by *O. lurida*, but did not report on the effects on the oysters.

### PREDATORS

Little study has been made of native invertebrate predators of *O. lurida*, because in commercial beds none are considered commercially significant. Several species of crabs, especially *Cancer productus* Randall, 1839, and *Cancer magister* Dana, 1852, can be serious pests on occasion in beds of relatively high salinity (Dimick et al. 1941, Quayle 1969, D. McMillin, pers. comm.), but rarely are in abundance near commercial beds. *Cancer gracilis* Dana, 1852, which is abundant over *O. lurida* beds, is capable of consuming adult *O. lurida* (Baker, 1988). The shore crab *Hemigrapsus oregonensis* (Dana, 1851) is thought to prey on juveniles (Dimick et al. 1941, Quayle 1969), but this has not been conclusively demonstrated. The large naticid snail *Polinices lewisii* (Gould, 1847) sometimes preys on adult *O. lurida*, and is regularly destroyed by oyster culturists, but it is not a major predator, in part because it seems incapable of penetrating dense *O. lurida* beds with its large, soft body and semi-burrowing mode of locomotion (Korringa 1976, D. McMillin, pers. comm.). The most serious native pests of adult *O. lurida* from the oyster culturists' viewpoint are several species of ducks (Aythyidae), including the white-winged scoter *Melanitta fusca* (Linné, 1758), the black scoter *Melanitta nigra* (Swainson, 1832), and the greater scaup *Aythya marila* (Linné, 1761), which are seasonally abundant predators (Galtsoff 1929, Sherwood 1931, D. McMillin, pers. comm.). In the Puget Sound, the starfish *Pisaster brevispinus* (Stimpson, 1857) can be a serious predator, but is not well-studied because it is easily controlled on diked beds (Galtsoff, 1929). Stingrays (*Myliobatus californica*) (Gill, 1865) are the most important oyster predator on soft bottoms in California (Townsend 1893, Roedel and Ripley 1950, Wicksten 1978), and leopard sharks *Triakis semifasciata* Girard, 1859, are also benthic bivalve predators in bays in California (Wicksten 1978).

Three introduced species have become serious predators on *O. lurida*. The Japanese oyster drill (a gastropod), *Cerastoma inornatum* (Récluz, 1851) (formerly *Ocenebra japonica*), was introduced in the late 1920s to the Puget Sound, and was soon considered the most serious predator of *O. lurida* (Chapman and Banner 1949). It preys by boring through the shell of small or adult *O. lurida*, and can eat one oyster per week (Chew 1958, 1960). As serious, if not more so, is the flatworm *Pseudostylochus ostreophagus* Hyman, 1955, also introduced from Japan, which bores through the shell of juveniles, and can cause up to 90% mortality of juveniles (Woelke 1956b). *P. ostreophagus* is reported only from the Puget Sound in North America, but *C. inornatum* is also found in various harbors of the Strait of Georgia, Boundary Bay, and the Strait of Juan de Fuca in British Columbia, in various bays and inlets of the Puget Sound and in Willapa Bay in Washington, in Netarts Bay in Oregon, and in Tomales Bay and Morro Bay in California (Carlton 1979). The eastern oyster drill, *Urosalpinx cinerea* (Say, 1822), has been introduced in many areas of the West Coast, including all of the sites mentioned for *C. inornatum* except for Netarts Bay, OR, and Morro Bay, CA, and has also been introduced near Crescent, British Columbia (Sherwood 1931), and in Humbolt Bay, San Francisco Bay, Elkhorn Slough, and Newport Bay, all in California (Carlton 1979). In Washington, *U. cinerea* has either died out or become very rare, and is not

considered a serious predator of *O. lurida* (D. McMillin, pers. comm.). Eley (1933) reported that *U. cinerea* destroyed 10–20% of juvenile *O. lurida* in Boundary Bay, British Columbia, and Bonnot (1938) stated that *U. cinerea* was abundant enough in Tomales Bay, CA, to halt an attempt there to culture *O. lurida*. *U. cinerea* remains abundant in California (Carlton 1979).

The native analogs of the introduced drills, the thalids *Nucella lamellosa* (Gmelin, 1792) and *Acanthina spirata* (Blainville, 1832) are not considered significant predators of *O. lurida*. Although both are reported to be minor predators on *O. lurida* (Bonnot 1935, Dimick et al. 1941), Kincaid (1957) believes that *O. lurida* benefits from *Nucella* preying on barnacles and mussels growing on and competing with *O. lurida*.

The anomuran shrimps *Callinassa californiana* Dana, 1854, and *Upogebia pugettensis* (Dana, 1852) are not predators of *O. lurida*, but are considered serious pests by oyster culturists. These shrimp kill the oysters by smothering them with their burrowing activity, by making the grounds too soft, and by draining diked areas through their burrows (Stevens 1928, Bonnot 1935, Dimick 1941).

### EPIBIONTS

*O. lurida* has the normal infestation of epizooans for a shell in the area, including; the barnacles *Balanus glandula* Darwin, 1854 and *Chthamalus dalli* Pilsbry, 1916 intertidally, and *Balanus crenatus* Bruguière, 1789 subtidally (Baker, unpubl. data); the mussels *Mytilus galloprovincialis* and *M. trossulus* (both formerly called *M. edulis*) (Miller and Morrison 1988, Baker, unpubl. data); the boring sponge *Cliona celata* Grant, 1826 in areas of higher salinity (Bonnot 1935); the sponge *Halicondria* sp.; various bryozoans; and various small polychaetes, including *Polydora* sp., which bore into the shells (pers. obs.). Dimick et al. (1941) concluded that smothering by an unknown species of bryozoan and by the tube-dwelling amphipod *Corophium spinecorne* Stimpson, 1957 were major sources of *O. lurida* juvenile mortality in Yaquina Bay, OR. The slipper shell, or "cup," *Crepidula fornicata* Linnaeus, 1785, introduced from the Atlantic, is very abundant in the Puget Sound, and at one time was considered a serious pest, but McKernan et al. (1949) concluded that there was no evidence that these were significant competitors of *O. lurida*. At subtidal sites, or in the pools created by dikes in the southwest Puget Sound, the introduced compound ascidians *Botryllus* sp. and *Botrylloides* sp. can completely cover *O. lurida* (R. Sardiña, pers. comm., pers. obs.). Galtsoff (1932) and Barrett (1963) stated that the Pacific oyster, *C. gigas* (Thunberg, 1795), is a serious competitor, but throughout the majority of *O. lurida*'s range this is not true, other than in the sense that culturists use former *O. lurida* beds to rear *C. gigas*. Oyster culturists, including Griffin (1941), report no evidence for superior competition by *C. gigas*, while Steele reported that *C. gigas* shells provide important hard substrate for *O. lurida*, and D. McMillin (Olympia Oyster Co., Shelton, WA, pers. comm.) believes that in its preferred habitat (see the section on habitat in this document) *O. lurida* out-competes *C. gigas*. In Humbolt Bay, *O. lurida* is considered to be a fouling pest on *C. gigas* (R. Sardiña, pers. comm.).

### HUMAN UTILIZATION

A major limitation to human utilization of *O. lurida* is its small size. It takes 1600–2000 shucked, raw *O. lurida* to fill one U.S. gallon, compared to 80–140 shucked *C. gigas* per gallon (McKee

1945), so *O. lurida* has been primarily a luxury item since the introduction of larger oysters to the Pacific coast of North America (Korringa 1976). Even at the high value of these oysters (\$250 per gallon of shucked meats in 1988), the labor cost is considered too high for a hatchery based industry, such as that for *C. gigas* (Donaldson 1988). The ancient Amerinds, however, made extensive use of *O. lurida*, based on shell midden excavations (Dall 1897, Kidd 1961, Elsasser and Heizer 1966, Iwamoto and Chew 1978, Barner 1981, R. Pullen, U.S.G.S., Coos Bay, OR, unpubl. data).

There is no record of commercial use of *O. lurida* in Alaska, and it is not at present considered to have commercial potential there (Wood 1973).

The commercial sale of *O. lurida* in historic times in British Columbia began in 1884, at an unspecified location (Quayle 1969). The harvest method was to rake together oysters at low tide on intertidal flats, apparently with no attempt to restock or manage the fishery (Stafford 1918), with the exception of Crescent Oyster Co., near Crescent, B.C., where a system of dikes were used to hold water at low tide (Sherwood 1931). This system is discussed for Washington state (below). Although many *O. lurida* harvest leases were taken out prior to World War II (Else 1933), there is no evidence that *O. lurida* in British Columbia became an important fishery. *Crassostrea virginica* (Gmelin, 1861) was cultured there starting in 1904 (Stafford 1917), and in the 1920s *C. gigas* was introduced and eventually became established (Quayle 1969). The success with these species probably distracted from attempts to manage *O. lurida*. By 1940, the *O. lurida* fishery had for all intents ceased (Quayle 1969), and in the British Columbia Department of Fisheries Oyster Bulletin (1950), Quayle remarked that no interest was shown in setting data published in the Bulletin for the previous year. There is no commercial fishery for *O. lurida* in British Columbia at present.

Washington has always been the center of the *O. lurida* industry. Large beds of *O. lurida* were discovered in Willapa Bay (then Shoalwater Bay) in 1850, and large shipments began that year to San Francisco, CA (Galtsoff 1929). By 1879 the regular trade to San Francisco had slowed (Barrett 1963), but the harvest continued to a high of 90,000 bushels in 1896, declining steadily thereafter (Galtsoff 1929), and completely ceasing by 1936 (Hopkins 1937). Figures by Ingersoll (1881) suggest a harvest high of 250,000 bushels in 1874. No attempts to manage the fishery were made in Willapa Bay (Galtsoff 1929). A similar trend befell the *O. lurida* beds in Samish Bay, in northern Washington. Harvesting began there about the same time as in Willapa Bay, and the population was effectively depleted in the 1890s (Steele 1964). An attempt was made a few years later to dike-culture *O. lurida* in Samish Bay, without success (Steele 1964).

The only significant culture of *O. lurida* took place in the southwest Puget Sound, WA, a network of narrow inlets with no major freshwater inputs. Populations in Budd Inlet were eradicated by 1891, apparently by pollution from the city and port of Olympia (Townsend 1893), but starting in about 1897 a system of dike culture was begun in inlets nearby, particularly parts of Eld Inlet, Totten Inlet, and Oakland Bay (Woelke 1956a). It has been stated that the dikes are modelled after a system developed in France (Kincaid 1928), but they only vaguely resemble the dike/pond systems in France (Milne 1972, Korringa 1976). The dikes in Washington are made of concrete, and are designed to enclose about an acre of intertidal flat so that it retains several inches of

water at all times (Galtsoff 1929). The dikes were plagued by settling and cracking (Hopkins 1937), and by the activities of burrowing anomuran shrimp (Stevens 1929), but apparently allowed better *O. lurida* survival and pest control (Galtsoff 1992). This system far exceeded any other bivalve culture efforts in North America for the period of 1890 to 1930 (Wallace 1966).

Oyster culturists either depended upon larvae from wild stocks for recruitment into the dikes, or purchased "seed" (shells with juveniles) from state owned beds in Oakland Bay, which they spread on the beds (Woelke 1956a). This allowed a fairly sustained yield at around 40,000 "sacks" (one sack equals 2 bushels, with about 2500 individuals per bushel) (Hopkins 1937) until about 1911, when most of the wild stocks were finally depleted. The industry in the southwest Puget Sound declined to about half that level, based entirely on cultivated *O. lurida*. The industry then persisted at that level until about 1927, when a large paper pulp mill on Oakland Bay went into operation, releasing sulfites either directly or indirectly into the inlet. Within several years *O. lurida* production in Oakland Bay was destroyed, including the "seed" beds, and overall production in the region began a slower decline. The pulp mill pollution was implicated in this decline (McKernan et al. 1949, Gunter and McKee 1960), although at about the same time 2 oyster predators were introduced from Japan; the gastropod *Cerastostoma inornatum* and the flatworm *Pseudostylochus ostreophagus*. *O. lurida* production declined steadily, so that by the late 1950s it had virtually ceased (Woelke 1956a). The pulp mill thought to be responsible shut down in 1956, but it was not until the early 1980s that *O. lurida* became commercially significant again (Kuons and Cardwell 1981, D. McMillin, pers. comm.). Now populations of the oyster thrive despite the introduced predators, and appear to be slowly gaining in commercial importance again in Eld Inlet, Totten Inlet, and Oakland Bay (Chew 1988).

*O. lurida* has been commercially harvested in Oregon from Netarts Bay, on a very small scale around the turn of the century and mainly for local consumption (Edmondson 1923), and from much larger beds in Yaquina Bay. The beds in Yaquina Bay were discovered in 1860, and were harvested steadily, without management, until largely depleted by 1930 (Fasten 1931). A series of experiments were made shortly after World War II in the culture of this species in Yaquina Bay (e.g., Breese 1953, Becker 1955), but today *O. lurida* is the least important species in shellfish culture in Yaquina Bay (Wachsmuth 1979). An attempt was made to introduce *O. lurida* to Coos Bay in 1914 (Edmondson 1923), without success (Galtsoff 1929). In 1987 an attempt was made to culture *O. lurida* in Winchester Bay, Oregon, but was abandoned within several years, although individuals continued to be brought in with *C. gigas* from Humboldt Bay, CA (R. Sardiña, pers. comm.).

Packard (1918), stated that *O. lurida* was commercially harvested from San Francisco Bay, in California, and Barret (1963) mentioned that it was harvested in 1859 from nearby Tomales Bay. In San Francisco Bay the shells of *O. lurida* were gathered for paving and for poultry feed, but Townsend (1893) considered this species a serious fouling pest on the introduced oyster *C. virginica*. In 1926 the *O. lurida* beds in Elkhorn Slough were heavily harvested, nearly wiping them out (Browning 1972), although small amounts were apparently taken in 1935 (Smith and MacKenzie 1948). In the early 1930s in Arcata Bay (the northern half of Humboldt Bay) several companies, in cooperation with the State of California and later Humboldt County, attempted to culture *O. lurida* there, using the dike system that was successful in the

southwest Puget Sound, WA. About 10 acres were eventually diked, and early results were encouraging (Bonnot 1936, 1937a, 1938), but very few oysters were actually marketed from there, and the various concerns soon lost interest in the project (Barrett 1963). At present there are large wild beds there that are marketed on a small scale (R. Sardiña, pers. comm.). Despite the abundance of *O. lurida* in some southern California inlets, it has apparently never been commercially harvested there.

There is no record known to this author of commercial harvest of either *O. lurida* or its sibling species, *O. conchaphila*, in Mexico.

With the exception of the introduced oyster drill, *C. inornatum*, and the flatworm *P. ostreophagus*, most of the major *O. lurida* predators can be controlled by shellfish culturists. Ducks are kept away by harassment (D. McMillin, pers. comm.), stingrays are kept out with low fences (Roedel and Ripley 1950), starfish are easily controlled in diked beds (Galtsoff 1929), and anururan shrimp can be controlled chemically or with deep wooden dikes (Galtsoff 1929). While the oyster drill *Cerastostoma* and the flatworm *Pseudostylochus* seem beyond control, they have not prevented a strong recovery by *O. lurida* in the southwest Puget Sound.

#### TAXONOMY NOTE

Harry (1985) considers *O. lurida* to be a junior synonym of *Ostreola* (*Ostrea*) *conchaphila* (Carpenter 1857), which had formerly been considered a tropical sibling species to *O. lurida*, and sympatric with it in southern California and the Baja peninsula, Mexico (Hertlein 1959). Hertlein (1959) provides evidence that

the 2 species grade into each other, and Abbott and Dance (1986) and Turgeon et al. (1988) have adopted Harry's revision. Harry's work was based only on adult shell and anatomy characteristics, however, and his generic reclassification, reminiscent of an attempted revision by Orton (1928), does not account for the genus *Tiostrea* (Chanley and Dinamani 1980), which is widely accepted in New Zealand and Chile. Although the evidence is strong for the conclusion that *O. lurida* and *O. conchaphila* at least grade together where sympatric, more work should be done before accepting the generic revision. Because the name *O. lurida* has been used in several hundred scientific papers and fishery publications, compared to very few scientific papers and no fishery publications for *O. conchaphila*, the specific name *lurida* should be retained. This is in accordance with the provisions of Article 79c: suppression of unused senior synonyms, in the International Code of Zoological Nomenclature, 3rd ed., adopted by the 20th General Assembly of the International Union of Biological Sciences. Couch and Hassler (1989), Arakawa (1990), Brock (1990), and Banks et al. (1993) have retained the name *O. lurida*.

The presently favored form of discrimination between similar species is molecular examination of the genetic structure, either in the nucleus or the mitochondria. Some work on cellular DNA content has been done on *O. lurida* by Hinegardner (1974). Wilkins (1976) examined genetic variability at 2 loci within *O. lurida* and other bivalve species, and Buroker (1982) has compared allozyme variation in *O. lurida* and two other *Ostrea* species. Brock (1990) used *O. lurida* in a comparison of genetic distances between 3 oyster genera, while Banks et al. (1993) used *O. lurida* as an out group to compare genetic relatedness of 2 species of *Crassostrea*.

#### *Ostrea Lurida* ANNOTATED BIBLIOGRAPHY

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- Valley, central California, and in the Pliocene-lower Pleistocene in the Tulare formation in the same region. This bulletin is incorporated into the following reference by Arnold and Anderson (1910), with no new information on *O. lurida*. Compare with Arnold (1903) and Howard (1935).]
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- marine organisms at the pier of the Scripps Institute of Oceanography, La Jolla, California. Bulletin of the Scripps Institute of Oceanography, University of California, Technical Series 3(3):37-86. [\*See also Coe (1930-1934), Coe and Allen (1937).]
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- Coe, W. R. & W. G. Allen. 1937. Growth of sedentary marine organisms on experimental blocks and plates for nine successive years. Bulletin of the Scripps Institute of Oceanography, Technical Series 4(4):101-136. [\*See also Coe (1930-1934).]
- Couch, D. & T. J. Hassler. 1989. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)—Olympia oyster. U.S. Fish & Wildlife Service Biological Report 82(11.124), 8 pp. [\*This is a summary of much of the important *O. lurida* literature.]
- Dall, W. H. 1897. Editorial correspondence: Marshfield, Oregon (Coos Bay) Aug. 23, 1897. *Nautilus* 11(6):66. [\*Dall mentions that *Ostrea lurida* is extinct in Coos Bay, Oregon, but is present in Indian shell middens. See also Carlton (1988).]
- Dall, W. H. 1914. Notes on west American oysters. *Nautilus* 28(1):1-3. [\*Carpenter's form *rufoides* of *Ostrea lurida* (1864) is long and thin and is found growing in currents, while the flattened form *expansa* (Carpenter, 1864a) is typical of specimens adhering to flat surfaces. See also Hertlein (1959).]
- Dall, W. H. 1921. Summary of the marine shellbearing mollusks of the northwest coast of America, from San Diego, California, to the Polar Sea, mostly contained in the collection of the United States National Museum, with illustrations of hitherto unfigured species. United States National Museum Bulletin 112. 215 pp.
- Davis, H. C. 1949. On cultivation of larvae *Ostrea lurida*. (Abstract). *Anat. Rec.* 105:111.
- Davis, H. C. 1950a. On interspecific hybridization in *Ostrea*. *Science* 111(2889):522. [\**Ostrea lurida* sperm will not fertilize or activate *Crassostrea virginica* eggs. See also Stafford (1913).]
- Davis, H. C. 1950b. On the culture of oyster larvae in the laboratory. National Shellfisheries Association Addresses. June 1949:33-38. [\*See also Davis (1961).]
- Davis, H. C. 1955. Mortality of Olympia oysters at low temperatures. *Biol. Bull.* 105(3):404-405. [\**Ostrea lurida* transplanted to Connecticut suffer 100% mortality in the winter, even when placed subtidally.]
- Davis, H. C. & P. E. Chanley. 1955. Effects of some dissolved substances on bivalve larvae. *Proc. Natl. Shellfish. Assoc.* 46:59-74. [\*This is a discussion of dissolved organic substances as nutrition.]
- Di Girolamo, R. G. 1970a. The uptake, elimination, and effect of processing on the survival of poliovirus in west coast oysters. Ph.D. Thesis, University of Washington, Seattle, Washington. 178 pp. [\*See Di Girolamo et al. (1970a, 1972, 1975).]
- Di Girolamo, R. G. 1970b. The uptake, elimination, and effect of processing on the survival of poliovirus in west coast oysters. Dissertation Abstracts 31B:821. [\*See Di Girolamo et al. (1970a, 1972, 1975).]
- Di Girolamo, R. G., J. Liston, J. R. Matches & A. K. Sparks. 1969. Viral accumulation and elimination by Pacific Coast shellfish. Research in Fisheries of the University of Washington School of Fisheries #300:72-74. [\*See Di Girolamo et al. (1970a, 1972, 1975).]
- Di Girolamo, R. G., J. Liston & J. R. Matches. 1970a. Survival of poliovirus in chilled, frozen, and processed oysters. *Appl. Microbiol.* 20(1):5863. [\*This discusses the uptake, presence, and elimination of poliovirus in *Ostrea lurida* and other oysters for human consumption. See also Di Girolamo et al. (1972, 1975).]
- Di Girolamo, R. G., J. Liston, J. R. Matches & A. K. Sparks. 1970b. Survival of poliovirus in chilled, frozen, and processed oysters. Research in Fisheries of the University of Washington School of Fisheries #320:43-44. [\*See Di Girolamo et al. (1970a, 1972, 1975).]
- Di Girolamo, R. G., J. Liston & J. R. Matches. 1972. Effects of irradiation on the survival of virus in west coast oysters. *Appl. Microbiol.* 24(6):1005-1006. [\*See also Di Girolamo et al. (1970a, 1975).]
- Di Girolamo, R. G., J. Liston & J. R. Matches. 1975. Uptake and elimination of poliovirus in west coast oysters. *Appl. Microbiol.* 29(2):260-264. [\*See also Di Girolamo et al. (1970a, 1972).]
- Dimick, R. E. & J. B. Long. 1939. Investigations of the native oyster in Yaquina Bay, Oregon, progress report 1, covering the period July 4 to September 15, 1939. Oregon Agricultural Experiment Station, Corvallis, Oregon, unpublished. [\*This and the following paper are the most important descriptive studies on the natural history of *Ostrea lurida* in Oregon. The second paper (Dimick et al. 1941.) summarizes the first, and goes into detail on hydrographic conditions, natural history, and population parameters. They are available at the Oregon State Library.]
- Dimick, R. E., G. England & J. B. Long. 1941. Native oyster investigations of Yaquina Bay, Oregon, progress report 2, covering the period July 4, 1939 to September 30, 1941. Oregon Agricultural Experiment Station, Corvallis, Oregon, unpublished. [\*See Dimick and Long (1939).]
- Donaldson, J. 1981. Hatchery rearing of the Olympia oyster. (Abstract). *J. Shellfish Res.* 1(1):131.
- Donaldson, J. 1988. Overview of an operating oyster hatchery. Proceedings of the 4th Alaska Aquaculture Conference. Alaska Sea Grant Report 88-4. pp. 77-81. [\*This article states that hatchery rearing of *O. lurida* is not cost-effective, given the present price of *O. lurida*.]
- Edmondson, C. H. 1920. Edible Mollusca of the Oregon Coast. Bernice Bishop Museum Occasional Papers Vol. 7, No. 9:179-201. [\**Ostrea lurida* was present in Yaquina and Netarts Bays, and in Coos Bay, where it had been introduced several years previously. Recruitment of *O. lurida* was reported in Coos Bay. This report also repeats the "old Indian story" that about 80 years previously (1840s), a large forest fire had led to the extinction of *O. lurida* in Coos Bay.]
- Edmondson, C. H. 1923. Shellfish resources of the Northwest Coast of the United States. Appendix III to Report of the U.S. Commissioner of Fisheries for 1922, Bureau of Fisheries Doc. #920. 21 pp. [\*This article is the best of the readily available early records of shellfish utilization on the Oregon coast, and should be read with Galtsoff (1929).]
- Eisenberg, J. M. 1981. *A Collector's Guide to Seashells of the World*. McGraw Hill Book Co., New York. 239 pp. [\**Ostrea lurida* is listed as Figure 15 on page 157, but the accompanying photograph is not of *O. lurida*. Figure 14 is probably *O. lurida*.]
- Elsasser, A. B. & R. F. Heizer. 1966. Excavation of two northwestern California sites. Report of the California Archaeological Survey #67: 1-151. [\**Ostrea lurida* shells were found to be a major part of an Indian shell midden near Patrick's Point, indicating a past population of *O. lurida* in that area.]
- Elsay, C. R. 1933. Oysters in British Columbia. *Bull. Biol. Board Can.* 34. 34 pp. [\*Included is the best discussion of *Ostrea lurida* culture in British Columbia, with a map of commercial sites. Most work was done in Boundary Bay and Ladysmith Harbor. It is stated that the introduced drill *Urosalpinx cinerea* destroys 10-20% of juvenile *O. lurida* in Boundary Bay. *Saxidomus nuttalli* Conrad 1837 (butter clam) shells were preferred (by culturists) *O. lurida* cultch.]
- Elsay, C. R. 1934. On the structure and function of the mantle and gills of *Ostrea gigs* (Thunberg) and of *Ostrea lurida* (Carpenter). Ph.D. Thesis, Rutgers University, New Jersey. 65 pp. (52 pages of unnumbered tables and figures). [\*See Elsay (1935).]
- Elsay, C. R. 1935. On the structure and function of the mantle and gill of *Ostrea lurida* and *Ostrea gigs*. *Trans. R. Soc. Can.* 48 (Section 5):131-160. [\*This is a careful anatomical study of *O. lurida* and includes good illustrations. It is taken from Elsay (1934).]
- Elston, R. A. 1990. *Mollusc Diseases: Guide for the Shellfish Farmer*. University of Washington Press, Seattle, WA. 73 pp. [\*On page 24, the author states that *Hexamita* "has been established" as a disease-causing agent in *Ostrea lurida*, citing Stein and Denison (1959). See annotation under Stein and Denison (1959), however.]
- Erickson, J. H. 1966. Bacteriological studies on commercial processing of Olympia oysters (*Ostrea lurida*). In: 1965 Proceedings of the Northwest Shellfish Sanitation Research Planning Conference. W. J. Beck & J. C. Hoff (eds.), pp. 23-31. U.S. Department of Health, Educa-

- tion, and Welfare Public Health Service Publication 999-FP-6. [\*This study shows how bacteria increases in processed *Ostrea lurida* stored at varying temperatures. See also Vasconcelos (1966a).]
- Farley, C. A. 1978. Viruses and virus-like lesions in marine mollusks. *Mar. Fish. Rev.* 40(1):18-20. [\*This and following articles mainly review previous literature, especially that of Mix et al. (1970-77) in reference to *Ostrea lurida*.]
- Farley, C. A. 1985. Viral gametocyte hypertrophy in oysters. International Council for the Exploration of the Sea; Identification Leaflets for Diseases and Parasites of Fish and Shellfish #25. 4 pp. [\*See Farley (1978).]
- Farley, C. A. 1988. Mass mortalities and infectious lethal diseases in bivalve mollusks and associations with geographic transfers of populations. (Abstract). *J. Shellfish Res.* 7(3):554. [\*Sarcoma epizootics cause "serious mortalities" in *Ostrea lurida* in Oregon.]
- Farley, C. A. & A. K. Sparks. 1970. Proliferative diseases of hemocytes, epithelial cells, and connective tissue cells in mollusks. *Bibliogr. Haematol.* 36:610-617. [\*See Farley (1978).]
- Farley, C. A., P. H. Wolf & R. a. Elston. 1988. A long-term study of "microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g.n.), and two new species, *Mikrocytos mackini* (sp.n.) and *Mikrocytos roughleyi* (sp.n.). *Fish. Bull.* 86(3):581-593. [\**Mikrocytos mackini* is found in *Crassostrea gigas*, *Ostrea edulis*, and *O. lurida*, where it is found in vesicular connective tissue, and causes abscess-like inflammatory lesions. In *C. gigas* and *O. edulis* it can cause high mortalities in British Columbia, and although no mortality studies were done for *O. lurida*, the 2 February samples out of 2 years (1969-70) of monthly samples from Yaquina Bay, Oregon had 24% and 12% infection rates in *O. lurida*. In other months there were no "microcells," but neoplastic lesions and *Mytilicola orientalis* infections were frequent.]
- Fasten, N. 1931. The Yaquina oyster beds of Oregon. *Am. Nat.* 45(700): 434-468. [\*This article is not as exhaustive as Dimick (1939) and Dimick (1941), but is much easier to acquire. It discusses aspects of the harvest, pollution, and ecology of *Ostrea lurida* in Yaquina Bay.]
- Filice, F. P. 1958. Invertebrates from the estuarine portion of San Francisco Bay and some factors influencing their distributions. *Wasmann J. Biol.* 1692:159-211. [\*A very few living *Ostrea lurida* were found in San Pablo Bay, within San Francisco Bay (compare to Packard 1918b), intertidally to 6 m in depth. Salinities were 18.0-19.6 ppt.]
- Fitch, J. E. 1953. Common marine bivalves of California. California Fish and Game Fish Bulletin #90. 102 pp. [\*Although often cited, this contains only a brief paragraph on *Ostrea lurida*, and no new information.]
- Freudenberg, W. 1934. Preliminary analysis of British Columbia oysters. Biological Board of Canada, Progress Reports of the Pacific Coast Stations #20:16-18. [\*As of 1932, *Ostrea lurida* apparently was still taken commercially from Victoria and Crescent harbors, and Ladysmith and Nanose Bays, in British Columbia.]
- Galtsoff, P. S. 1929. Oyster industry of the Pacific coast of the United States. Appendix VIII to Report of the U.S. Commissioner of Fisheries for 1929, Bureau of Fisheries Doc. #1066:367-400. [\*This is a comprehensive article on oyster culture of that period, and includes some information on enemies of *O. lurida* in the Puget Sound that is rarely reported elsewhere. It should be read in conjunction with Edmondson (1923).]
- Galtsoff, P. S. 1932. Introduction of Japanese oysters into the United States. Bureau of Fisheries Fishery Circular #12. 16 pp. [\*Galtsoff feared that *Crassostrea gigas* would become an important competitor of *Ostrea lurida* (contrast to Griffin 1941, and Steele 1957).]
- Galtsoff, P. S. 1949. The oyster and oyster industry of the United States. U.S. Fish and Wildlife Service Fishery Leaflet 187. 10 pp. [\*This updates Galtsoff (1929) with regard to the Pacific coast oyster industry.]
- Galtsoff, P. S. 1964. The American Oyster. *Fish. Bull.* 64:1-480. [\*Various aspects of *Ostrea lurida* reproduction and ecology are compared to those of *Crassostrea virginica*, but most of the document is about *C. virginica*.]
- Gibson, G. G. 1974. Oyster mortality study summary report 1966-72. Fish Commission of Oregon, Management and Research Division: Newport, Oregon. 37 pp. [\*This includes several studies of physiological tolerances of *Ostrea lurida*.]
- Gilbert, C. H. 1891. Report upon certain investigations relating to the planting of oysters in southern California. *Bull. U.S. Comm. Fish.* 9:95-98. [\*Along with Townsend (1893), this is one of the most important discussions of early *Ostrea lurida* distribution in southern California.]
- Glude, J. B. 1948. Oyster investigation. Washington Department of Fisheries 1947 Annual Bulletin. pp. 17-20. [\*This bulletin and other Washington Department of Fisheries publications contained brief mention of *Ostrea lurida* culture into the 1960s, tracking the decline and temporary collapse of the dike culture industry. See also Glude et al. (1946), Lindsay et al. (1948-1958), Westley (1959-1963), and Woelke (1956-1959).]
- Glude, J. B. 1975. A summary report of Pacific coast oyster mortality investigations 1965-1972. Proceedings of the Third U.S.-Japan Meeting on Aquaculture, 1974. Special Publication of the Japanese Fishery Agency and the Japan Sea Regional Fisheries Research Laboratory: pp. 1-28. [\*This article is mostly about *Crassostrea gigas* but briefly discusses the research to date on neoplastic disorders in *Ostrea lurida*.]
- Glude, J. B., V. Tartar & R. Tollefson. 1946. Review of recent spawning and setting seasons. Washington Department of Fisheries Olympia Oyster Bulletin. 4 pp.
- Gooding, D. & R. N. Ward. 1953. Shellfish research. Washington Department of Fisheries 62nd Annual Report. pp. 72-81. [\*See Glude (1948).]
- Grant, U. S., IV & H. R. Gale. 1931. Catalogue of the Marine Pliocene and Pleistocene Mollusca of California and Adjacent Regions. Memoirs of the San Diego Society of Natural History 1. 1036 pp.
- Griffin, E. 1941. *Oysters Have Eyes, or The Travels of a Pacific Oyster*. Wilberilla Publishers, Seattle, Washington. 53 pp. [\*This is mainly about *Crassostrea gigas* culture in Willapa Bay, Washington, and only briefly mentions *Ostrea lurida* exploitation (pp. 5-6). On page 43, Griffin states that the shells of *Crassostrea gigas* provide important settlement space for *O. lurida* juveniles. Contrast with Galtsoff (1932).]
- Gunter, G. 1950. The generic status of living oysters and, the scientific names of the common American species. *Am. Mid. Nat.* 43(2):438-499. [\*This discusses generic differences between *Crassostrea* and *Ostrea*.]
- Gunter, G. & G. McKee. 1960. On oysters and sulfite waste liquor. Special Consultants' Report to the Washington Pollution Control Commission. 93 pp. [\*This is a comprehensive report on all aspects of pulp mill pollution and oysters in the southwest Puget Sound, Washington, but relies heavily on other work, especially Hopkins et al. (1935), McKernan et al. (1949), Odlaug (1949), and Stein et al. (1959a). The biological conclusions are that pulp mill pollution is deleterious, if not outright toxic, to *Ostrea lurida*.]
- Haderlie, E. C. & D. P. Abbott. 1980. Bivalvia. In: *Intertidal Invertebrates of California*. R. H. Morris, D. P. Abbott & E. C. Haderlie (eds.). pp. 355-411. Stanford University Press, Stanford, California. [\*On page 364 is a short but excellent review of the natural history of *Ostrea lurida*.]
- Harry, H. W. 1985. Synopsis of the supraspecific classification of living oysters (Bivalvia: Gryphaeidae and Ostreidae). *Veliger* 28(2):121-158. [\*In this paper, Harry has revised most oyster taxonomy at the genus and species level. *Ostrea lurida* is declared a junior synonym of *Ostreola* (formerly *Ostrea*) *conchaphila* (Carpenter 1857), with a range of Alaska to Panama. The American Fisheries Society (Turgeon et al. 1988) and Abbott and Dance (1986) have adopted this revision, but authors of other recent publications have not.]
- Harry, H. W. 1986. The relevancy of the generic concept to the geographic distribution of living oysters (Gryphaeidae and Ostreidae). *Am.*

- Malacol. Bull.* 4(2):157-162. [\*The author presents an apparent correlation of oyster generic relatedness with geography, but this scheme depends on his own extensive generic revisions (Harry 1985).]
- Hedgepeth, J. W. & S. Obrebski. 1981. Willapa Bay: a historical perspective and a rationale for research. Fish and Wildlife Service Biological Services Program AFWS/OBS-81/03. 60 pp. [\*This article briefly records the history of *Ostrea lurida* harvests in Willapa Bay.]
- Henderson, J. 1935. Fossil non-marine Mollusca of North America. Geological Society of America Special Papers #3. 313 pp. [\*This work is a compilation of studies by other authors; in the case of *O. lurida*, Arnold (1909) and Arnold and Anderson (1910).]
- Hertlein, L. G. 1959. Notes on California oysters. *Veliger* 2(1):5-10. [\*Included is a discussion of *Ostrea lurida* and *O. conchaphila* in southern California. See also Dall (1914).]
- Hinegardner, R. 1974. Cellular DNA content of the Mollusca. *Comp. Biochem. Physiol.* 47A(2):447-460. [\*The cellular DNA of 110 mollusks, including *Ostrea lurida*, was measured. For *O. lurida*, the DNA content is 1.3 picograms per cell, near the median for mollusks.]
- Hoff, J. C. & R. C. Becker. 1961. The accumulation and elimination of crude and clarified poliovirus suspensions by shellfish. *Am. J. Epidemiol.* 90(1):53-61. [\**Ostrea lurida* depurated itself of filtered poliovirus to nondetectable levels within 96 hours but initially accumulates more of and takes longer to depurate itself of crude poliovirus. See also Di Girolamo et al. (1970a, 1972, 1975).]
- Holmes, H. B. 1927. An investigation of sawdust pollution in relation to oysters in Yaquina Bay. U.S. Bureau of Fisheries, unpublished.
- Holway, T. W. 1934. Some observations on the Pacific oyster "*Crassostrea gigas*" Thunberg and the native oyster "*Ostrea lurida*" Carpenter in Willapa Bay. M.S. Thesis, University of Washington, Seattle, Washington. 62 pp. [\*The natural history of *Ostrea lurida* is discussed here.]
- Hopkins, A. E. 1935a. Attachment of larvae of the Olympia oyster, *Ostrea lurida*, to plane surfaces. *Ecology* 16(1):82-87. [\*This, and Hopkins (1936b, 1937), are the most important studies on the natural history and physiology of *Ostrea lurida* in the Puget Sound, and also contain information about oyster culture at that time.]
- Hopkins, A. E. 1935b. Temperature and the shell movements of oysters. *Bull. U.S. Bur. Fish.* 47:1-14. [\*See Hopkins (1935a).]
- Hopkins, A. E. 1936a. Activity of the adductor muscle in oysters. *Physiol. Zool.* 9(4):498-507. [\*See Hopkins (1935a).]
- Hopkins, A. E. 1936b. Ecological observations on spawning and early larval development in the Olympia oyster (*Ostrea lurida*). *Ecology* 17(4):551-566. [\*See Hopkins (1935a).]
- Hopkins, A. E. 1936c. Pulsating blood vessels in the oyster. *Science* 83(2163):581. [\*See Hopkins (1935a).]
- Hopkins, A. E. 1936d. Pulsation of blood vessels in oysters, *Ostrea lurida* and *O. gigas*. *Biol. Bull.* 70(3):413-425. [\*See Hopkins (1935a).]
- Hopkins, A. E. 1937. Experimental observations on spawning, larval development, and setting in the Olympia oyster *Ostrea lurida*. *Bull. U.S. Bur. Fish.* 48:438-503. [\*See Hopkins (1935a).]
- Hopkins, A. E., P. S. Galtsoff & H. C. McMillin. 1935. Effects of pulp mill pollution on oysters. *Bull. U.S. Bur. Fish.* 47:125-162. [\*This article is written as 3 separate articles within 1 document (by each of the 3 authors above), which are often referenced separately.]
- Hori, J. 1933. On the development of the Olympia oyster, *Ostrea lurida* Carpenter, transplanted from the United States of Japan. *Bull. Jpn. Soc. Sci. Fish.* 1(6):269-276. [\*This is a careful study of spawning and development of *Ostrea lurida*.]
- Howard, P. J. 1935. Report on Buena Vista Hills, a portion of the Midway Sunset oil field. *Calif. Oil Fields* 20:5-22. [\*Included are tables of common fossils of this area, in the southwest part of the San Joaquin Valley, California. *Ostrea lurida* is common in both estuarine and marine deposits, apparently dating back to the early Pliocene.]
- Human, V. L. 1971. The occurrence of *Urosalpinx cinerea* in Newport Bay. *Veliger* 13(3):299. [\**Ostrea lurida* is present at Newport Bay, California, as small, scattered individuals.]
- Hutchinson, E. N. & B. M. Brennan. 1936. Oyster culture: a natural resource revived. Published by the State of Washington Secretary of State. 14 pp.
- Imai, T., S. Sakai, H. Okeda & T. Yoshida. 1954. Breeding of the Olympia oyster in tanks and culture experiments in Japanese waters. *Tohoku J. Agric. Res.* 5(1):13-25. [\**Ostrea lurida* spawning under laboratory conditions and in the field in Japan is discussed.]
- Ingersoll, E. 1881. *History and Present Condition of the Fisheries Industry: The Oyster Industry*. U.S. Government Printing Office, Washington, D.C. 250 pp. [\**Ostrea lurida* is mentioned on pp. 201-202 as the Shoalwater Bay oyster. Commercial harvest in Shoalwater (Willapa) Bay began in 1850, climbed to 250,000 bushels in 1874, and declined to 15,000 bushels in 1880.]
- Iversen, E. S. 1968. *Farming the Edge of the Sea*. Fishing News Books Ltd., London, G.B. 301 pp. [\**Ostrea lurida* dike culture is mentioned briefly.]
- Iwamota, R. N. & K. K. Chew. 1978. Skokomish intertidal shellfish survey. Research in Fisheries of the University of Washington School of Fisheries #480:73. [\**Ostrea lurida* is reported in Indian shell middens in northern Washington.]
- Johnson, A. G., F. M. Utter & K. Noggol. 1972. Electrophoretic variants of aspartate aminotransferase and adductor muscle proteins in the native oyster (*Ostrea lurida*). *Anim. Blood Groups Biochem. Genet.* 3(2):109-113.]
- Jones, E. J. & A. K. Sparks. 1969. An unusual histopathological condition in *Ostrea lurida* from Yaquina Bay, Oregon. (Abstract). *Proc. Natl. Shellfish. Assoc.* 59:11.
- Keen, A. M. 1937. *An Abridged Checklist and Bibliography of West North American Marine Mollusca*. Stanford University Press, Stanford, California. 84 pp. [\*This is taken mainly from Dall (1921).]
- Keen, A. M. 1944. Check list of California tertiary marine Mollusca. Geological Society of America, Special Paper #56. 280 pp. [\*This is compiled from various other sources, such as Howard (1935). Over 30 fossil *Ostrea* species are listed.]
- Keen, A. M. 1962. A new West Mexican subgenus and new species of Montacutidae (Mollusca: Pelecypoda), with a list of Mollusca from Bahia de San Quintin. *Pac. Nat.* 3(9):321-328. [\*This includes mention of *Ostrea lurida* in Baja California (Mexico).]
- Keep, J. 1891. Mollusks of the San Francisco markets. *Nautilus* 4(9):97-100. [\*The "native, or Oregon oyster" was found in small numbers in seafood markets at this time, and the name suggest that they were taken from either Yaquina Bay (Oregon) or Willapa Bay (Washington).]
- Keep, J., rev. by J. L. Baily, Jr. 1935. *West Coast Shells*. Stanford University Press, Stanford, California. 350 pp.
- Kidd, R. S. 1967. The Martin site, southwestern Washington. *Tebiwa* 10:13-30. [\**Ostrea lurida* shells make up about 70% of the shells of this American Indian food preparation midden on the west shore of Willapa Bay, Washington.]
- Kincaid, T. B. 1928. Development of oyster industry in the Pacific. *Trans. Am. Fish. Soc.* 58:117-122. [\*This briefly discusses the decline of the *Ostrea lurida* industry, and mentions that the dike system is copied from the French, but this statement could not be independently confirmed. See also Korringa (1976).]
- Kincaid, T. B. 1951. *The Oyster Industry of Willapa Bay, Washington*. Calliostoma Co., Seattle, Washington. 45 pp. [\*This non-technical publication includes a brief history of *Ostrea lurida* utilization in Willapa Bay, and a photograph of *O. lurida* larvae.]
- Kincaid, T. B. 1957. *Local Races and Clines in the Marine Gastropod *Thais lamellosa**. Calliostoma Co., Seattle, Washington. 75 pp. [\**Nucella lamellosa* is not a significant predator on *Ostrea lurida*, but mainly consumes mussels and barnacles, potential competitors of the oyster (p. 33).]
- Kolbe, E. R., M. J. English & J. R. Miner. 1979. Oyster production in the Pacific Northwest. Oregon Agricultural Experiment Station Technical Paper #5211.
- Korringa, P. 1952. Recent advances in oyster biology. *Q. Rev. Biol.* 27(3):266-308 and 27(4):339-365. [\*This article is printed in 2 parts in 2 issues of this journal. *Crassostrea* is called *Gryphaea*. "Recent"

- means post-1940 for the most part, which was after the majority of the *Ostrea lurida* research, but there are occasional references to *O. lurida*.]
- Korringa, P. 1976. *Farming the Flat Oysters of the Genus Ostrea*. Elsevier Scientific Publishing Co., New York. 238 pp. [\*Most of 1 chapter is devoted to *Ostrea lurida* culture in Washington State. There is also a description of intertidal "parks" and holding ponds for the culture and harvest of *O. edulis* in France, from which the dike system in Washington may have evolved, in part.]
- Kozloff, E. N. 1973. *Seashore Life of the Northern Pacific Coast*. University of Washington Press, Seattle, Washington, 370 pp. [\*This is a popular, rather than scientific document. It states that *Ostrea lurida* is a common fouling organism in some parts of San Francisco Bay.]
- Kozloff, E. N. 1974. *Keys to the Marine Invertebrates of Puget Sound, the San Juan Archipelago, and Adjacent Regions*. University of Washington Press, Seattle, Washington. 266 pp.
- Kuons, R. R. & R. D. Cardwell. 1981. Significant areas for certain species of food fish and shellfish in Puget Sound. Washington Department of Fisheries Technical Report 59. 49 pp. [\*This paper discusses present and potential *Ostrea lurida* culture areas.]
- Kvenvolden, K. A., D. J. Blunt & H. E. Clifton. 1979. Amino acid racemization in quaternary shell deposits at Willapa Bay, Washington, USA. *Geochim. Cosmochim. Acta* 43(9):1505-1520.
- Lindsay, C. E. 1948. Oyster investigations. Washington Department of Fisheries 1948 *Annual Report*. pp. 41-44. [\*This bulletin and other Washington Department of Fisheries publications contained brief mention of *Ostrea lurida* culture into the 1960s, tracking the decline and temporary collapse of the dike culture industry. See also Glude et al. (1946), Lindsay et al. (1948-1958), Westley (1959-1963), and Woelke (1956-1959).]
- Lindsay, C. E. & D. C. McMillin. 1948. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series VII (1-12). [\*This and following references for Lindsay et al. are series of short (1-10 pp.) mimeographs meant for circulation among oyster culturists and researchers. These present collected data on *Ostrea lurida* settlement and mortalities in the southwest Puget Sound, but rarely summarize or discuss it.]
- Lindsay, C. E. & D. C. McMillin. 1949. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series VIII (1-14). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E., D. C. McMillin, S. E. Sayce & H. E. Wiksten. 1950. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series IX (1-14). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E. & S. E. Sayce. 1951. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series X (1-16). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E., C. E. Woelke, R. E. Westley & H. E. Wiksten. 1952. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series XI (1-14). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E., R. E. Westley & C. E. Woelke. 1953. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series XII (1-4). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E., R. E. Westley & C. E. Woelke. 1954. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series XIII (1-14). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E., R. E. Westley & C. E. Woelke. 1955. Washington Department of Fisheries Puget Sound Oyster Bulletin, Series XIV (1-11). [\*See Lindsay and McMillin (1948).]
- Lindsay, C. E., R. E. Westley & S. E. Sayce. 1958. Prediction of oyster setting in the State of Washington. *Proc. Natl. Shellfish. Assoc.* 49: 59-70. [\*The authors describe methods used to predict the magnitude of *Ostrea lurida* settlement in the southwest Puget Sound. See also Lindsay and McMillin (1948).]
- Lindsay, C. E., C. E. Woelke & R. E. Westley. 1956. Preliminary report on Olympia oyster survival experiment: Oakland Bay and North Bay, October 1953-March 1956. Washington Department of Fisheries Shellfish Lab. 7 pp. [\*This reports on the effect of pulp mill pollution on *Ostrea lurida*.]
- Loosanoff, V. L. & H. C. Davis. 1963. Rearing of bivalve mollusks. *Adv. Mar. Biol.* 1:1-136. [\*Included here is a description of several stages of the larvae of *O. lurida*.]
- Loosanoff, V. L., H. C. Davis & P. E. Chanley. 1966. Dimensions and shapes of larvae of some marine bivalve mollusks. *Malacologia* 4(2): 351-435. [\*Included are pictures of *Ostrea lurida* larvae throughout development.]
- Loosanoff, V. L. & R. R. Marak. 1951. Culturing lamellibranch larvae. *Anat. Rec.* 111:129-130.
- MacGinitie, G. E. 1935. Ecological aspects of a California estuary. *Am. Mid. Nat.* 16(5):629-765. [\**Ostrea lurida* was abundant in Elkhorn Slough on artificially-placed substrate, especially iron, to 8 km upstream from the mouth.]
- MacGinitie, G. E. 1941. On the method of feeding of four pelecypods. *Biol. Bull.* 80(1):18-25. [\*The author replaced part of 1 valve of 4 species of Pacific coast bivalves, including *Ostrea lurida*, with a glass plate, and observed the filter feeding mechanism. In all 4 species the feeding method was nearly identical.]
- McKee, L. G. 1945. Planting and marketing oysters in the Pacific Northwest. U.S. Fish & Wildlife Service Fishery Leaflet 52. 6 pp. [\*It takes 1600-2000 *Ostrea lurida* bodies to fill a gallon, compared to 80-140 *Crassostrea gigas* bodies.]
- McKernan, D. L., V. Tartar & R. Tollefson. 1949. An investigation of the decline of the native oyster industry of Washington, with special reference to the effects of sulfite pulp mill waste on the Olympia oyster (*Ostrea lurida*). Washington Department of Fisheries Biological Report #49A:115-165. [\*This was a critical pollution study for *O. lurida* in the southwest Puget Sound, and also contains a study on the effects of *Crepidula fornicata* on *Ostrea lurida*, and a discussion of other oyster pests.]
- Mackin, J. G. 1971. Oyster culture and disease. *Proceedings of the First Annual Workshop, World Mariculture Society*. pp. 35-38. [\*The author states that *Ostrea lurida* suffered heavy mortalities from a haplosporidium disease in southern Puget Sound, Washington, but does not cite a reference for this.]
- McLachlan, D. H. & J. Ayers. 1979. *Fieldbook of Pacific Northwest Sea Creatures*. Naturegraph Publishers, Inc., Happy Camp, California. 208 pp. [\*There is a color photograph of *Ostrea lurida* on page 123.]
- Marchand, M. & F. Cabane. 1980. Hydrocarbures dans les moules et les huitres. *Rev. Int. Oceanogr. Méd.* 59:3-30. (In French with English summary.)
- Marriage, L. D. 1954. The bay clams of Oregon: their economic importance, relative abundance, and general distribution. Oregon Fish Commission Contribution 20, 47 pp. [\*In 1954, *Ostrea lurida* was apparently present but rare in Netarts Bay, present in Yaquina Bay, and absent in all other Oregon estuaries.]
- Matthiessen, G. C. 1970. A review of oyster culture and the oyster industry in North America. Woods Hole Oceanographic Institute Contribution 2528, Woods Hole, Massachusetts. 55 pp. [\*This reviews the status of the U.S. and Canada oyster industry, including *Ostrea lurida*, during the 1960s.]
- Menzel, R. W. 1968. Chromosome number in nine families of marine pelecypod mollusks. *Nautilus* 82(2):45-50 and plates on pp. 53-58.
- Miller, W., III & S. D. Morrison. 1988. Marginal marine Pleistocene fossils from near mouth of Mad River, northern California. *Proc. Calif. Acad. Sci.* 45(10):255-266. [\*A well preserved late Pleistocene "oyster garden" contains, in decreasing order of frequency; *Ostrea lurida* in clumps on a mud bottom; the ectoparasitic gastropod *Odosstomia nota* Dall and Bartsh, 1909; *Balanus* sp. (on *O. lurida* shells); the infaunal clam *Protothaca staminea* (Conrad, 1937); the snail *Bitonium eschrichtii* (Middendorf, 1849); and 10 less common species of mollusks. Also common in *O. lurida* shells were bore holes of a polychaete, *Polydora* sp.; and a sponge, *Cliona* sp. A bryozoan, a brachyuran crab, seagrass, and fish bones were also preserved. Com-

- plete with a sketch of the reconstructed community, this is one of the best estuarine ecological studies of an *Ostrea lurida* community.]
- Milne, P. H. 1972. *Fish and Shellfish Farming in Coastal Waters*. Fishing News Books, London. 208 pp. [\*The discussion here on intertidal oyster farming in France does not describe anything very similar to the dikes used for *O. lurida*.]
- Mix, M. C. 1974. Diseases of shellfish in Yaquina Bay, Oregon. (Abstract). *Proc. Natl. Shellfish. Assoc.* 64:14. [\*See Mix (1975a).]
- Mix, M. C. 1975a. Proliferative characteristics of atypical cells in native oysters (*Ostrea lurida*) from Yaquina Bay, Oregon. *J. Invertebr. Pathol.* 26(3):289–298. [\*This and following articles by Mix et al. deal mainly with neoplastic disorders of *O. lurida*, which do not seem to be significant on a population level. See also Jones and Sparks (1969), Farley and Sparks (1970), and Sparks (1970).]
- Mix, M. C. 1975b. The neoplastic disease of Yaquina Bay mollusks. In: *The Cell Cycle in Malignancy and Immunity. Proceedings of the 13th Annual Hanford Biology Symposium*, J. C. Hampton (ed.). pp. 369–386. National Technical Information Service, Springfield, Virginia. [\*See Mix (1975a).]
- Mix, M. C. 1976a. A general model for leucocyte cell renewal in bivalve mollusks. *Mar. Fish. Rev.* 38(1):37–41. [\*This includes a brief discussion of leucocyte genesis in *Ostrea lurida* and 2 photographs of leucocytes. See also Mix (1975a).]
- Mix, M. C. 1976b. A review of the cellular proliferative disorders of oysters (*Ostrea lurida*) from Yaquina Bay, Oregon. *Prog. Exp. Tumor Res.* 20:275–282. [\*See Mix (1975a).]
- Mix, M. C., H. J. Pribble, R. T. Riley & S. P. Tomasovic. 1977. Neoplastic diseases in bivalve mollusks from Oregon with emphasis on research on proliferative disorders in Yaquina Bay oysters. *Ann. N.Y. Acad. Sci.* 298:356–373. [\*This paper reports heavy mortalities of *Ostrea lurida* in the late 1950s and early 1960s in Yaquina Bay, Oregon, but concludes that neoplastic disorders are not responsible. This is contrary to the conclusions of Sparks et al. (1970).]
- Mix, M. C. & R. T. Riley, 1977. A pericardial tumor in a native (Olympia) oyster, *Ostrea lurida*, from Yaquina Bay, Oregon. *J. Invertebr. Pathol.* 30(1):104–107. [\*See Mix (1975a).]
- Mix, M. C. & V. Sprague. 1970. Occurrence of a haplosporidium in native oysters (*Ostrea lurida*) from Yaquina Bay and Alsea Bay, Oregon. *J. Invertebr. Pathol.* 23(2):252–254. [\**O. lurida* was introduced to Alsea Bay, Oregon, for this study.]
- Modin, J. C. 1969. Pesticide concentrations in California bays and estuaries. Proceedings of the Symposium on Mollusca, Cochim, India, Part II. pp. 519–530. [\*This reports on the concentrations of organochlorine pesticide residues (DDT, DDD, DDE) in the tissue of *Ostrea lurida* and *Crassostrea gigas* in 6 estuaries in 1967 and 1968. This does not state whether *O. lurida* was tested from all 6 estuaries. Pesticide levels were low in Humbolt Bay, Tomales Bay, and Drakes Estero (mostly less than 15 parts per billion), higher in San Francisco Bay and Morro Bay (mostly 30–100 ppb), and highest in Elkhorn Slough (mostly 150–400 ppb). The highest level was 920 parts per billion for a June 1967 sample in Elkhorn Slough.]
- Moore, H. F. 1898. Oysters and methods of oyster culture. Report of the U.S. Commissioner of Fisheries for 1897. 263–340. [\*This includes a short discussion of *Ostrea lurida* dike culture.]
- Morris, P. A. 1966. *A Field Guide to Shells of the Pacific Coast and Hawaii, Including Shells of the Gulf of California*, 2nd ed. Houghton Mifflin Co., Boston. 297 pp. [\*Reprinted 1980 as *Pacific Coast Shells*.]
- Morris, R. W. 1948. Experiments of the larval culture of the native oyster, *Ostrea lurida* Carpenter, M.S. Thesis, Oregon State College, Corvallis, Oregon. 46 pp. [\*This was the earliest of 5 M.S. theses on the culture of *Ostrea lurida* out of Oregon State College during this time period. See also Becker (1955), Breese (1953), Pasquale (1953), and Warren (1951).]
- Mudie, P. J., B. M. Browning & J. W. Speth. 1974. The natural resources of Los Penasquitos Lagoon and recommendations for use and development. California Department of Fish and Game Coastal Wetlands Series #7: 96 pp. [\*Prior to the dredging of the mouth, no marine shellfish existed in the lagoon, but within 1 year of dredging, at least 20 marine species, including *Ostrea lurida*, had become established. Compare to Bradshaw et al. (1976).]
- Nelson, T. C. 1928. On the distribution of critical temperatures for spawning and for ciliary activity in bivalve Mollusca. *Science* 67(1730):220–221. [\*This reviews *Ostrea lurida* research on the effects of temperature.]
- Nightengale, H. W. 1936. *Red Tide Organisms: Their Occurrence and Influence on Marine Aquatic Animals with Special Reference to Shellfish in the Waters of the Pacific Coast*. The Argus Press, Seattle, Washington. 24 pp.
- Nosho, T. 1989. Small scale oyster farming for pleasure and profit. Washington Sea Grant Aquaculture Series WSG-AS 89-1, 12 pp. [\*This contains a good description of the biology of *Ostrea lurida* in layman's terms, and mentions that it takes 300 to make a pint of shucked meat.]
- Odlaug, T. O. 1946. The effect of the copepod, *Mytilicola orientalis*, upon the Olympia oyster, *O. lurida*. *Trans. Am. Microsc. Soc.* 65(4): 311–317. [\*This is the most comprehensive discussion of this parasite in *Ostrea lurida*.]
- Odlaug, T. O. 1949. Effects of stabilized and unstabilized waste sulphite liquor on the Olympia oyster, *O. lurida*. *Trans. Am. Microsc. Soc.* 68(2):163–182.]
- Oldroyd, I. S. 1924. *The Marine Shells of the West Coast of North America. Pelecypoda and Brachiopoda*. Stanford University Press, Stanford, California. 247 pp. [\*This book reproduces the mistakes of earlier authors, but it contains the first paragraph of Carpenter's hard-to-acquire original description (see also Palmer 1958).]
- Oldroyd, I. S. 1925. Marine shells of Puget Sound and vicinity. *Publications of the Puget Sound Biological Station* 4:1–271. [\*\*\*"Vicinity" includes to the Queen Charlotte Islands in northern British Columbia. This merely lists species collected by the author and others, with the sites collected. *Ostrea lurida* is reported at Blakely Island, in the San Juan Archipelago in Washington, and Van Dormop Creek on Vancouver Island, British Columbia, among other sites.]
- Orcutt, C. R. 1922. Mollusks dredged from San Diego Bay. *Nautilus* 36(1):33–34. [\**Ostrea lurida* makes up a major portion of the dredged shells.]
- Orcutt, H. C. 1958. California oyster ground utilization plan. *Proc. Natl. Shellfish. Assoc.* 49:98–100. [\**Ostrea lurida* is not regarded as having commercial significance in California by the California Department of Fish and Game (see also Barrett 1963), but beds of native oysters are protected from development nonetheless.]
- Orton, J. H. 1928a. The dominant species of *Ostrea*. *Nature* 121(3044): 320–321. [\*The author recognizes basic differences within the genus, and proposes breaking it up into *Monoeciostria* (*Ostrea*) and *Dieciostraea* (*Crassostrea*, *Saccostrea*), and renaming the species. *O. lurida* would become *M. vancouverensis*.]
- Orton, J. H. 1928b. Transplantation of the European oyster. *Science* 67(1745):582–583. [\*Includes a mention of the differences discussed in Orton (1928a).]
- Packard, E. L. 1918a. A quantitative analysis of the molluscan fauna of San Francisco Bay. *Univ. Calif. Publ. Zool.* 18(13):299–336. [\**Ostrea lurida* was reported at many stations, but dead material was included. It was reported living just outside the mouth of the bay, and on shells on all substrates in the bay except sand. *Ostrea elongata* referred to here is *Crassostrea virginica*.]
- Packard, E. L. 1918b. Molluscan fauna from San Francisco Bay. *Univ. Calif. Publ. Zool.* 14(2):199–452. [\*Despite the apparent journal volume discrepancy with Packard (1918a), this is the correct citation; Volume 14(1) was published in 1914, but 14(2) was not published until 1918, after Volumes 15–18 had been issued. This is a report of one of the voyages of the U.S. government research vessel *Albatross* in and about San Francisco Bay, and mainly reports distribution of the species in the bay. Living *Ostrea lurida* is reported throughout the southern part of the bay, north to Pt. Richmond, intertidally to 4 fathoms in depth. Dead shells were also found in the middle of San Pablo Bay, and

- material (not specified as living or dead) were found between the mouth of the bay and the Farallon Islands, out to sea. The author also mentions that *Ostrea* is harvested commercially for human consumption in the southern part of the bay.]
- Palmer, K. V. W. 1945. Molluscan types in the Carpenter Collection in the Redpath Museum. *Nautilus* 58(3):97-100. [\*The type specimen of *Ostrea lurida*, from Shoalwater (Willapa) Bay, Washington, is in the Peter Redpath Museum, McGill University, Montreal, Canada. This was confirmed by personal communication from Ms. Susan Gabe, Curator, in 1991.]
- Palmer, K. V. W. 1958. Type Specimens of Marine Mollusca Described by P. P. Carpenter from the West Coast (San Diego to British Columbia). *Geol. Soc. Am. Mem.* 76. 376 pp. [\*This contains the second paragraph of Carpenter's original species description for *O. lurida* (see also Oldroyd 1924).]
- Pasquale, N. 1953. Rearing of the native oyster larvae, *Ostrea lurida* Carp., in concrete and wooden tanks under controlled conditions. M.S. Thesis, Oregon State College, Corvallis, Oregon. 74 pp. [\*See also Becker (1955), Breese (1953), Morris (1948), and Warren (1951).]
- Paul, A. J. & H. M. Feder. 1976. Clam, mussel, and oyster resources of Alaska. *Univ. Alaska Inst. Mar. Sci. Rep.* 76-4:41 pp. [\*This is an independent, if brief, confirmation of the existence of *O. lurida* in southeast Alaska. No sites given; *O. lurida* said to be present "but seldom encountered in dense aggregations there."]
- Peters, E. C. 1988. Recent investigations on the disseminated sarcomas of marine bivalve mollusks. *Am. Fish. Soc. Spec. Publ.* 18:74-92. [\*Review of Mix (1974-1976), Mix et al. (1970-1977), and Sparks (1970). This paper uses *Ostreola conchaphila* for *Ostrea lurida* (see Harry 1985).]
- Pibbs, F. D. 1969. Larval culture of bivalve mollusks. (Abstract). *Proc. Natl. Shellfish. Assoc.* 59:12.
- Pibbs, F. D. 1970. Laboratory hatching and rearing of Pacific coast clams and oysters. Completion report for period July 1976-June 1970. Fish Commission of Oregon for U.S. National Marine Fisheries Service. NOAA COM-75-10568. 43 pp. Available from National Technical Information Service. [\*Only the first of 3 progress reports within this document contain any information about *Ostrea lurida*. It takes 13 days to reach a larval shell length of 250  $\mu$  at 16-20°C.]
- Quayle, D. B. 1941. The edible mollusca of British Columbia. *Prov. British Columbia Rep. Comm. Fish.* 1940:75-87. [\**Ostrea lurida* populations are reported to occur in inlets near the mouths of streams in British Columbia. This material is also found in Quayle (1960).]
- Quayle, D. B. 1949. *British Columbia Department of Fisheries Oyster Bulletin* 1:1-12. [\*These contain brief mention of *Ostrea lurida* settlement in British Columbia.]
- Quayle, D. B. 1960. *The Intertidal Bivalves of British Columbia*. British Columbia Provincial Museum Handbook Series, #17. 104 pp. [\*See Quayle (1941).]
- Quayle, D. B. 1969. Pacific oyster culture in British Columbia. *Fish. Res. Board Can. Bull.* 169. 192 pp. [\*There is a good discussion of the shell and anatomy of *Ostrea lurida* in the process of comparing it to *Crassostrea* in the first chapter.]
- Ranson, G. 1950. La chambre promyaire et la classification zoologique des Ostreides. *J. Conchyliol.* 90:195-200. (In French). [\**Ostrea* lacks a promyal chamber, *Crassostrea* has it.]
- Ranson, G. 1960. Les prodissochonques (coquilles larvaires) des Ostreides vivants. *Bull. Inst. Oceanogr.* #1183. 41 pp. (In French).
- Richard, G. P. 1988. Microbial purification of shellfish: a review of depuration and relaying. *J. Food Prot.* 51(3):218-251. [\*Depuration is the process whereby edible shellfish rid themselves of contaminants, and relaying is the transport of these shellfish from contaminated growing areas to depuration sites. The *Ostrea lurida* material, on pages 231-233, is taken from Hoff and Becker (1961), Beck et al. (1966), and Di Girolamo et al. (1975).]
- Ricketts, E. F. & J. Calvin, revised by J. W. Hedgpeth. 1952. *Between Pacific Tides*. Stanford University Press, Stanford, California. 516 pp. [\*This includes a brief discussion of *Ostrea lurida* in the chapter entitled "Bay and estuary: rocky shores."]
- Rosenfield, A. 1969. Oyster diseases in North America and some methods for their control. In: *Artificial Propagation of Commercially Valuable Shellfish*. K. S. Price, Jr. & D. L. Maurer (eds.). pp. 67-78. University of Delaware, Newark, Delaware. [\*Mentions that *Hexamita* species are ubiquitous, but does not state that they are pathogenic (see Stein and Denison 1959).]
- Roughley, T. C. 1929. Monoecious oysters. *Nature* 124(3134):793. [\**Ostrea* (now *Tiostrea*) *lutaria* and *O.* (now *Saccostrea*) *culcullata* are classified together as hermaphroditic oysters, along with *O. lurida*. This character is no longer a basis for taxonomic status, however.]
- Santos, J. M., S. L. Downing & K. K. Chew. 1992a. The effects of water temperature on sexual development of adult Olympia oysters. *O. lurida*. (Abstract). *J. Shellfish Res.* 11(1):206-207. [\*See Santos et al. (1992b).]
- Santos, J. M., S. L. Downing & K. K. Chew. 1992b. The effects of water temperature on sexual development of adult Olympia oysters, *Ostrea lurida*. (Abstract). *J. Shellfish Res.* 11(2):556. [\*The effects of water temperature on gametogenesis, spawning, and reproductive output are summarized. This is essentially the same abstract as Santos et al. (1992a), but with more complete results.]
- Scofield, N. B. 1928. Oysters in California. *Calif. Fish Game* 14(3):203-240. [\*The author mentions minor attempts to culture *Ostrea lurida* in California, but no localities are given. See Bonnot (1932-1938).]
- Scofield, N. B. 1932. Commercial fishery notes: oyster growing in California. *Calif. Fish Game* 18(1):63-64. [\*The initiation of *Ostrea lurida* culture experiments in Humbolt Bay is described (see also Bonnot 1932-1938), and earlier experiments are mentioned.]
- Sherwood, H. P. 1931. The oyster industry of North America: a record of a brief tour of some of the centres on the Atlantic and Pacific coasts, and of a summer in Canada. *J. Cons.* 6(3):361-386. [\*Non-scientific, but containing much historical information. This is one of the few references to the *Ostrea lurida* dikes near Crescent, B.C., and to the fact that oysters were sometimes transferred for "fattening." It also mentions that *Urosalpinx cinerea* were abundant at Crescent, as a result of *Crassostrea virginica* introductions, and that several species of ducks are major *O. lurida* predators.]
- Sindermann, C. J. 1970. The role and control of diseases in mariculture. In: *Food-Drugs from the Sea Proceedings*, 1969. H. E. Youngken, Jr. (ed.). pp. 145-173. Marine Technology Society, Washington, D.C. [\*See Mix (1975a) and Odlaug (1946).]
- Sindermann, C. J. 1977. *Mytilicola* disease of oysters. pp. 215-216. In: *Disease Diagnosis and Control in North American Marine Aquaculture*. C. J. Sindermann (eds.). Developments in Aquaculture and Fisheries Science 6. [\*See Mix (1973s) and Odlaug (1946).]
- Smith, A. G. & G. MacKenzie, Jr. 1948. The marine mollusks and brachiopods of Monterey Bay, California, and vicinity. *Proc. Calif. Acad. Sci.* 4th Ser. 26:147-245. [\**Ostrea lurida* is reported as "fairly common" subtidally in Monterey Bay, a full marine environment. The authors also report that *O. lurida* was harvested commercially on a small scale in Elkhorn Slough in 1935.]
- Smith, L. S. 1955. Observations on the polyclad *Pseudostylochus ostreophagus*. University of Washington M.S. Thesis, Seattle, Washington. [\*This is probably the major source of information for Woelke's paper (1956c).]
- Smith, M. 1907. Annotated list of the Mollusca found in the vicinity of La Jolla, San Diego Co., Cal. *Nautilus* 21(5):55-59. [\**O. lurida* found growing on stones in False Bay. See also Coe (1932b).]
- Sparks, A. K. 1963. Survey of the Oyster Potential of Hawaii. *Hawaii Dep. Land Nat. Res. Div. Fish Game*. 44 pp. [\*See Brock (1960).]
- Sparks, A. K. 1970. Tumors and tumorlike conditions in invertebrates. In: *Invertebrate Pathology: Noncommunicable Diseases*. A. K. Sparks (ed.). pp. 271-371. Academic Press, New York. [\*On pages 349-355 there is a discussion of neoplastic disease of *Ostrea lurida* in Yaquina Bay, Oregon, taken from Jones and Sparks (1969) and Farley and Sparks (1970).]

- Sparks, A. K., K. K. Chew, S. C. Katkansky, D. E. Weitcamp, E. J. Robbins & L. Schwartz. 1967. Epizootics in experimental marine shellfish populations. Research in Fisheries of the University of Washington School of Fisheries #240:35-37. [\*This and the following articles briefly discuss *Ostrea lurida* disease and parasites.]
- Sparks, A. K., K. K. Chew, D. E. Weitcamp, E. J. Jones & L. Schwartz. 1968. Epizootics in experimental marine shellfish populations. Research in Fisheries of the University of Washington School of Fisheries #280:33-34.
- Sparks, A. K., D. E. Weitcamp & E. J. Jones. 1970. Oyster mortality investigations. Research in Fisheries of the University of Washington School of Fisheries #320:26-27.
- Stafford, J. 1912. Supplementary observations on the development of the Canadian oyster. *Am. Nat.* 46(1):29-40. [\*This mentions that *Ostrea lurida* is found in large numbers in Nanoose Bay and Oyster Harbor. The eggs of *O. lurida* are larger than those of *Crassostrea*.]
- Stafford, J. 1913. Conservation of the oyster. Province of British Columbia Report of Commercial Fisheries, 1912. pp. 71-80. [\*This is mostly about *Crassostrea virginica* in British Columbia, but Stafford also mentions that several attempts to crossbreed this species with *Ostrea lurida* failed. See also Davis (1950a).]
- Stafford, J. 1914. The native oyster of British Columbia (*Ostrea lurida*, Carpenter). Province of British Columbia Report of Commercial Fisheries, 1913. pp. 79-102. [\*This and the following articles by Stafford are exhaustive studies of *Ostrea lurida* in British Columbia, but much of the work is contradicted by Hopkins (1935a, 1936b, 1937). There are some good drawings of *O. lurida* larvae and juveniles in this article, and some internal anatomy drawings in the following article (1915), along with an extensive biogeographical study. The other articles contain some doubtful physiological studies and some harvest information.]
- Stafford, J. 1915. The native oyster of British Columbia (*Ostrea lurida*, Carpenter). Province of British Columbia Report of Commercial Fisheries 1914. pp. 100-119. [\*See Stafford (1914).]
- Stafford, J. 1916. The native oyster of British Columbia (*Ostrea lurida*, Carpenter). Province of British Columbia Report of Commercial Fisheries 1915. pp. 141-160. [\*See Stafford (1914).]
- Stafford, J. 1917. The native oyster of British Columbia (*Ostrea lurida*, Carpenter). Province of British Columbia Report of Commercial Fisheries, 1916. pp. 88-120. [\*See Stafford (1914).]
- Stafford, J. 1918. The native oyster of British Columbia (*Ostrea lurida*, Carpenter). Province of British Columbia Report of Commercial Fisheries 1917. pp. 91-112. [\*See Stafford (1914).]
- Steele, E. N. 1957. *The Rise and Decline of the Olympia Oyster*. Fulco Publications, Elma, Washington. 126 pp. [\*Despite some biases and writing style peculiarities, this is the best single reference for the history and cultivation of *O. lurida* in the southwest Puget Sound, the industry center. It goes into considerable depth on the politics of the Olympia oyster industry, and places most of the blame for the industry collapse in the 1950s on pulp mill pollution. Steele also believes that *Crassostrea gigas* poses no biological threat to *O. lurida* (contrast to Galtsoff 1932).]
- Steele, E. N. 1964. *The Immigrant Oyster*. Warren's Quick Print, Olympia, Washington. 179 pp. [\*In the short chapter entitled "Cultivation and harvesting of oysters" there is apparently the only reference to the fate (overharvesting) of the large *Ostrea lurida* beds in Samish Bay, northern Washington.]
- Stein, J. E., G. M. Clark, J. G. Denison & R. E. Petersen. 1959a. The decline of the Olympia oysters (*O. lurida*) for the period of June 1957 through March 1959. Report of the Olympic Research Division, Rayonier, Inc., Shelton, Washington. 36 pp. [\*This, Stein and Petersen (1959) and Stein et al. (1959b) are important studies for Gunter and McKee's paper (1960), but are unfortunately hard to come by. Rayonier, Inc., the owner of a large pulp mill, was the defendant in legal cases regarding pollution in the southwest Puget Sound.]
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## GROWTH OF BLACK ABALONE, *HALIOTIS CRACHERODII* LEACH, AT SAN MIGUEL ISLAND AND POINT ARGUELLO, CALIFORNIA

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**ABSTRACT** Black abalone (*Haliotis cracherodii*) growth studies were done at 3 intertidal locations on San Miguel Island and at a mainland site, Point Arguello, in southern California from 1986 to 1990. We compared growth, determined from tag-recapture, among sites. Data were analyzed using likelihood methodology, including nonlinear least squares for parameter estimation, likelihood ratio test for hypothesis testing, and the von Bertalanffy growth model. Growth varied both by location and year, which was a possible effect of a lethal disease called withering syndrome (WS). At sites that were apparently unaffected by WS, growth was optimum and similar. A discussion of growth relevant to future recovery of black abalone populations is presented.

**KEY WORDS:** Black abalone (*Haliotis cracherodii*), growth, tag-recapture, withering syndrome

### INTRODUCTION

Black abalone, *Haliotis cracherodii* Leach, are gastropod molluscs inhabiting rocky intertidal areas from Oregon to southern Baja California, Mexico, including the Channel Islands off southern California. This species may grow to a size of 197 mm, but are usually 125 to 150 mm in length. Minimum recreational and commercial harvest sizes are 127 and 146 mm, respectively. Distribution patterns and size made this abalone important economically for coastal dwellers from prehistoric times to the present. Coastal native Americans traditionally used black abalone for food, decoration, and tools. Modern coastal inhabitants used them mostly for food (Cox 1962).

Black abalone were not important in the post-World War II commercial abalone fishery until the late 1960s. Subtidal red and pink abalone, *H. rufescens* and *H. corrugata*, were more desirable species, and were plentiful along the central California coast and the Channel Islands.

Sea otters are efficient predators on abalones. Recovery and expansion of the sea otter population in central California dislocated the fishery there to other otter-free areas, mostly the Channel Islands in southern California. As increased harvesting pressure reduced availability of red and pink abalone in southern California, interest turned to the abundant black abalone in the intertidal zone.

Regulation changes in 1968 that allowed the export of abalone to a strong Asian market soon propelled black abalone landings to high levels. It exceeded 861.8 metric tons (M.T.) in 1973. A daily harvest limit and increased minimum harvest size were established in 1974. Between 1974 and 1986 landings ranged between 158.7 M.T. and 204.1 M.T., but dropped to 90.7 M.T. by 1989 (Parker et al. 1992). Commercial landings declined to 17.4 M.T. in 1992 as mortality from withering syndrome devastated black abalone stocks throughout southern California (Haaker 1994). Both recreational and commercial fisheries were closed in 1993 to protect remaining stocks.

Several studies have dealt with black abalone growth. Leighton and Boolootian (1963) conducted studies of black abalone at 2 coastal locations in southern California. Wright (1975) studied *in*

*situ* growth of black abalone at Santa Cruz Island and found that growth was "extremely variable". She measured abalone from the spire to the anterior edge of the shell, which made her growth determinations difficult to compare with other studies. Blecha et al. (1992) conducted a black abalone growth study at Diablo Canyon on the central California mainland from 1979 to 1984. They calculated growth using long term field studies with tag-recapture methods at 4 locations.

In this paper we report our growth study at San Miguel Island and Point Arguello in southern California. While previous studies had collected growth information at various locations, they did not include enough locations within the area of the black abalone fishery, which encompassed both warm subtropical and temperate waters.

More locations with growth information were needed for setting management strategies and evaluating their effectiveness. This would include determining yield-per-recruit and egg-per-recruit models, finding relations between recruitment and breeding stocks, and estimating mortality of pre-harvest cohorts (Day and Flemming 1992).

Our purpose was to determine growth at several locations within the commercial fishery. The original number of locations was greater, but withering syndrome devastated the prospective sites in southern California before useful data could be collected. Point Arguello was added later as part of a black abalone monitoring program.

### METHODS

Black abalone are particularly well suited to growth rate measurements because they are accessible in the intertidal zone. Often, we can make successive annual measurements on the same individuals, sometimes without removing the animal from the substrate.

We used tag and recapture methods to obtain annual growth increment data for black abalone at 4 intertidal locations: Otter Harbor, Harris Point and Crook Point on San Miguel Island, and Point Arguello on the mainland coast (Fig. 1). The island sites were adjacent to intertidal monitoring sites in the Channel Islands

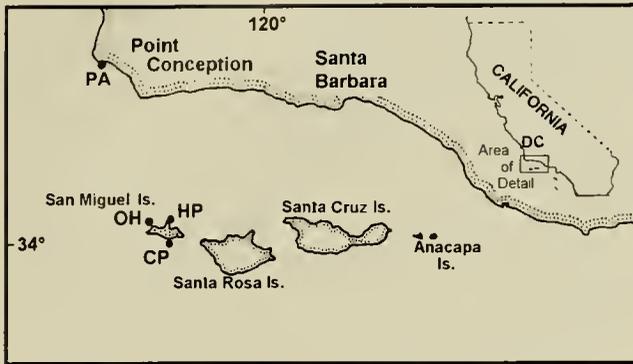


Figure 1. The location of growth sites. OH, Otter Harbor; CP, Crook Point; HP, Harris Point, at San Miguel Island; PA, Point Arguello, near Point Conception; and DC, Diablos Canyon, north of Point Conception (inset).

National Park, and the mainland site was on a remote part of Vandenberg Air Force Base, at a location closed to public access.

The Otter Harbor site is on the northwest side of San Miguel Island, at the east end of Simonton Cove ( $\approx 34^{\circ}03.1'N$ ,  $120^{\circ}24.5'W$ ). The site is exposed to the north, and is unprotected from northerly and northwesterly swells and winds. A long low reef is offshore. The reef, which is exposed at low tide, attenuates the force of waves. The actual locations of the tagged abalone were on broad flat rock and in crevices 1 to 2 m wide, which paralleled the coast.

The Harris Point site is near the northernmost point of San Miguel Island ( $\approx 34^{\circ}04.3'N$ ,  $120^{\circ}21.6'W$ ). This site is open to the northwest and often receives the force of large swells and surf. The site is a small cove with large unmovable boulders and flat rock substrate with crevices and small caves. The center of the site is composed of a rocky substrate with some movable boulders.

The Crook Point site is west of Crook Point on the south side of San Miguel Island ( $\approx 34^{\circ}01.0'N$ ,  $120^{\circ}22.2'W$ ). It faces south and includes a rocky substrate with large, mostly unmovable boulders, shallow tidepools, and crevices in the substrate. The tag site is completely dry at low tide. Extending seaward, a pair of rocky reefs directs waves and surf onto the site.

The Point Arguello area ( $\approx 34^{\circ}33.5'N$ ,  $120^{\circ}37.8'W$ ) contains the

southernmost of the large black abalone populations on the mainland, and is outside the commercial harvest area. It faces west, and is exposed to heavy waves and surf. The site is in an area of rocky reefs and tidepools. Much of the site is dry at low tide. Many undercuts and crevices, and some movable boulders are present.

We visited the sites during low tide at nearly yearly intervals. We visited the island sites in March 1986 and 1987, April 1988, and March 1989. We visited Point Arguello in November 1988 and 1989, February 1990 (a continuation of the November 1989 visit), and November 1990 (Table 1A,B). Black abalone were first tagged at Otter Harbor and Harris Point in 1986, and at Crook Point in 1987. Since visits were annual, seasonal growth patterns were not considered.

We removed black abalone from the rocks, and tagged them with numbered stainless steel washer tags wired through the shell pores with stainless steel wire. The tags are durable and cause no harm to the abalone (Haaker et al. 1986). We measured the abalone (maximum shell length) to the nearest mm, and replaced them.

On subsequent visits, we measured and replaced as many tagged abalone as we could find. We tagged additional abalone to maintain numbers of tagged abalone at each site (Table 1). Only abalone that could be removed without injury or habitat disruption were used. Smaller, cryptic abalone were not usually available. Abalone smaller than about 50 mm were too small to carry the tag and were not included.

We analyzed the data using the likelihood methodology of Kimura (1980). Kimura used nonlinear least squares for parameter estimation, and the likelihood ratio test for hypothesis testing. His approach was consistent with the recommendation to avoid the Ford-Walford line for parameter estimation. Galucci and Quinn (1979), and Vaughan and Kanciruk (1982) discussed the superiority of the nonlinear method. They recommended that it replace the Ford-Walford line for the von Bertalanffy growth curve.

We used nonlinear least squares to estimate the parameters of a standard von Bertalanffy growth model for mark-recapture data (Kirkwood and Somers 1984). Using the equation of Kirkwood and Somers, the von Bertalanffy growth equation is:

$$d = (L_{\infty} - r) (1 - e^{-Kt})$$

where  $d$  is change in length between marking and recapture, and  $r$

TABLE 1.

Numbers of black abalone tagged, their size range, number recaptured, and recapture rate, by location and year at A: San Miguel Island and B: Point Arguello.

A. Location	1986		1987			1988			1989		Total		
	Tag n	Size range	Tag n	Recap. n	Size range	Tag n	Recap. n	Size range	Recap. n	Size range	Tag n	Recap. n	Recap. %
Otter Harbor	271	61-160	58	97	102-134	75	61	51-147	60	55-145	404	218	54.0
Harris Point	518	62-156	97	143	53-144	107	158	50-144	106	66-142	722	407	56.4
Crook Point	0	-	45	0	55-99	93	6	60-104	33	63-142	138	39	28.3
Total	789		221	240		275	225		199		1264	664	52.5
B. Location	1988		1989		1990		Total						
	Tag n	Size range	Tag n	Size range	Recap n	Tag n	Recap. n	Recap. %					
Point Arguello	200	47-170	199	57-159	79	399	105	26.3					

Tag n is number of abalone tagged, recap. n is number of tagged abalone recaptured, and size range is in mm.

is length at marking. The von Bertalanffy parameters are mean asymptotic length  $L_\infty$  and the Brody growth coefficient  $K$ . The SAS nonlinear least squares program NLIN was used for parameter estimation and hypothesis testing (SAS Institute, Inc. 1988). A statistical significance level of 0.05 was used for this study.

For analysis, we omitted outliers in the data. Outliers were determined by inspecting graphs of residuals from the growth curves. We considered as outliers data with residuals that were clearly separated from the rest of the graph. With outliers removed, the parameter estimates for a site were often more consistent with growth estimates for other sites. We removed a single outlier from each of the following data: 1988 Otter Harbor data, 1986 and 1988 Harris Point data, and 1990 Point Arguello data.

Using the likelihood ratio test, we compared years for a given location. The 3 hypotheses tested were: common  $K$ , common  $L_\infty$ , and common  $K$  and  $L_\infty$ . The equations for the hypotheses are given in Table 2A. A hypothesis of a common  $K$  assumed that a single value of  $K$  was satisfactory across years for a given location; no assumption was made about  $L_\infty$ . A hypothesis of a common  $L_\infty$  assumed that a single value for  $L_\infty$  was satisfactory across years for a given location; no assumption was made about  $K$ .

A hypothesis of a common  $K$  and  $L_\infty$  assumed that both a single  $K$  and a single  $L_\infty$  were satisfactory across years for a given location. This inferred that no year effect was present. Thus, a single von Bertalanffy curve could be plotted for a given location. This test of a common  $K$  and  $L_\infty$  was used for reporting comparisons among years for a given location.

Similarly, we compared locations for a given year using the likelihood ratio test. The 3 hypotheses tested were: common  $K$ , common  $L_\infty$ , and common  $K$  and  $L_\infty$ . The equations for the hypotheses are given in Table 2B. A hypothesis of a common  $K$  assumed that a single value of  $K$  was satisfactory across locations for a given year. A hypothesis of a common  $L_\infty$  assumed that a single value for  $L_\infty$  was satisfactory across locations for a given year. A hypothesis of a common  $K$  and  $L_\infty$  assumed that both a single  $K$  and a single  $L_\infty$  were satisfactory across locations for a given year. This test of a common  $K$  and  $L_\infty$  was used for reporting comparisons among locations for a given year.

Using the results of comparing years for a location and the parameter estimates, we plotted the von Bertalanffy growth curves. Where the test of a common  $K$  and  $L_\infty$  across years for a given location was not statistically significant, all the yearly data were used to plot a single growth curve for that location. Otherwise, a different growth curve was plotted for each year at that location.

We did some additional tests when we added Point Arguello, a mainland site, to the comparisons. An overall test checked for any differences among the 4 sites: Point Arguello and the 3 island sites. Then pairwise comparisons were made between Point Arguello and each of the island sites. Also, Otter Harbor with its combined yearly data was compared with Point Arguello with its combined yearly data.

In reporting long term growth, we followed Blecha et al. (1992), in using age at  $L_\infty - 1$ . When  $L_\infty$  was rounded to a single decimal place, age was ambiguous with respect to  $L_\infty$ ; that is, we could not assign a single age to a rounded value of  $L_\infty$ . Correcting  $L_\infty$  by  $-1$  usually eliminated this ambiguity.

**RESULTS**

A total of 1,263 black abalone, ranging in size from 51 to 160 mm, was tagged at 3 locations on San Miguel Island. Of these,

**TABLE 2.**

**Null hypotheses and associated equations.**

**A: Comparison of years within location**

- Let  $d_{ij}$  = change in length for the  $j$ th abalone in the  $i$ th year,
- $r_{ij}$  = length at marking for the  $j$ th abalone in the  $i$ th year,
- $L_{\infty i}$  = mean asymptotic length for the  $i$ th year,
- $K_i$  = Brody growth coefficient for the  $i$ th year,
- $i = 1, \dots, I$ , where  $I$  is the number of years compared.

The null hypotheses, and equations for  $d_{ij}$  are:

- Common  $K$ :  $K_1 = \dots = K_I = K, d_{ij} = (L_{\infty i} - r_{ij})(1 - \exp[-Kt])$
- Common  $L_\infty$ :  $L_{\infty 1} = \dots = L_{\infty I} = L_\infty, d_{ij} = (L_\infty - r_{ij})(1 - \exp[-K_i t])$
- Common  $K$ , common  $L_\infty$ :  $K_1 = \dots = K_I = K, L_{\infty 1} = \dots = L_{\infty I} = L_\infty, d_{ij} = (L_\infty - r_{ij})(1 - \exp[-Kt])$

The  $\chi^2$  statistic is:

$$\chi_r^2 = -N \log(RSS_f/RSS_h)$$

- where  $r$  = degrees of freedom (df),
- $RSS_f$  = residual sum of squares for full parameter model,
- $RSS_h$  = residual sum of squares for null hypothesis,
- $N$  = sample size.

**B. Comparison of locations within year**

- Let  $d_{ij}$  = change in length for the  $j$ th abalone in the  $i$ th location,
- $r_{ij}$  = length at marking for the  $j$ th abalone in the  $i$ th location,
- $L_{\infty i}$  = mean asymptotic length for the  $i$ th location,
- $K_i$  = Brody growth coefficient for the  $i$ th location,
- $i = 1, \dots, I$ , where  $I$  is the number of locations compared.

The null hypotheses, equations for  $d_{ij}$ , and  $\chi^2$  statistic look the same as in Table 2A.

664 were recaptured at least once, for a recapture rate of 52.6 percent (Table 1).

The results of the likelihood ratio tests comparing the years 1986, 1987, and 1988 for a given location on San Miguel Island are presented in Table 3. No year comparisons were made for Crook Point due to insufficient data (Table 1). Table 4 contains results of the likelihood ratio tests comparing locations on San Miguel Island for a given year. The von Bertalanffy parameter estimates are stratified by location in Table 5, and by year in Table 6.

Growth at Otter Harbor was the same from year to year ( $p = 0.32$ , common  $K$  and  $L_\infty$ ) (Table 3). Thus, the yearly data were combined for plotting a single von Bertalanffy curve (Fig. 2). The parameter estimates for the curve are listed in Table 5. The  $L_\infty$  parameter by itself did not change at Otter Harbor during the study ( $p = 0.21$ , common  $L_\infty$ ) (Table 3).

Black abalone at Otter Harbor had some of the highest  $L_\infty$  and

TABLE 3.

Likelihood ratio tests comparing years at a given location for black abalone at Otter Harbor, Harris Point on San Miguel Island, and Point Arguello.

Location	n	Hypothesis	RSS	df	$\chi^2$	p-val
Otter Harbor	164	Full parameters	1352.06			
		Common $K$	1360.52	2	1.02	0.60
		Common $L_\infty$	1378.21	2	3.14	0.21
		Common $K$ and $L_\infty$	1391.15	4	4.67	0.32
Harris Point	285	Full parameters	715.82			
		Common $K$	717.04	2	0.48	0.79
		Common $L_\infty$	751.31	2	13.79	0.001
		Common $K$ and $L_\infty$	753.93	4	14.78	0.005
Pt. Arguello	83	Full parameters	517.14			
		Common $K$	518.14	1	0.05	0.82
		Common $L_\infty$	519.08	1	0.21	0.65
		Common $K$ and $L_\infty$	519.09	2	0.21	0.90

The  $n$  is sample size,  $RSS$  is residual sum of squares,  $df$  is degrees of freedom,  $\chi^2$  is Chi-square value, and  $p$ -val is probability.

$K$  values. Of the sites at San Miguel Island, we also found the largest individuals, 160 mm, at Otter Harbor (Table 5). The estimated age at  $L_\infty - 1$  was 27 years. The estimated time to recreational size, 127 mm, was 17 years. We could not determine the time to commercial size, 146 mm.

Growth at Harris Point was different from year to year ( $p = 0.005$ , common  $K$  and  $L_\infty$ ) (Table 3). Thus, separate growth curves were plotted for each year, using parameter estimates in Table 5 (Fig. 2). The estimated  $L_\infty$  declined in each succeeding year, from 119.6 in 1986–87, to 110.9 in 1987–88, and to 102.1 mm in 1988–89 (Table 5). This decline in  $L_\infty$  was statistically significant, and showed that growth at Harris Point declined with time ( $p = 0.001$ , common  $L_\infty$ ) (Table 3). None of the  $L_\infty$  exceeded the recreational size limit, 127 mm.

At Harris Point, estimated age at  $L_\infty - 1$  for the 3 periods were 79, 93, and 92 years, respectively, which were the highest for this study. The  $K$  parameters for the 3 periods were consistent, but low, ranging from 0.06 for the first period, to 0.05 thereafter ( $p = 0.79$ , common  $K$ ) (Tables 3 and 5). These  $L_\infty$  and  $K$  parameters were the lowest of any sites, including Point Arguello, and together with the age at  $L_\infty - 1$ , showed that this site did not support optimum black abalone growth. The results in Tables 4A and 5 showed that growth at Harris Point was slower than at Otter Harbor.

An insufficient number (6 abalone) was measured in 1987 from Crook Point to estimate growth, but more data (28 abalone) were available in 1988 to derive parameter estimates. The estimates for both  $L_\infty$  and  $K$  had large standard errors, compared with other sites (Table 5). Thus, we did not plot a growth curve for Crook Point. The estimate for  $L_\infty$  at Crook Point was the highest for this study. Nonetheless, we disregarded this result, since the parameter estimate had an unacceptably high standard error (Table 5).

At mainland Point Arguello, a total of 399 black abalone, ranging in size from 47 to 159 mm, was tagged. Of these, 105 were recaptured at least once, for a recapture rate of 26.3 percent (Table 1).

The results of the likelihood ratio tests that compared the years 1988 and 1989 at Point Arguello are presented in Table 3. Growth at Point Arguello did not change during that period ( $p = 0.90$ ,

common  $K$  and  $L_\infty$ ) (Table 3). Thus, the yearly data were combined to plot a single von Bertalanffy curve in Figure 2 from the parameter estimates in Table 5.

As stated earlier, we disregarded the  $L_\infty$  parameter at Crook Point, due to its high standard error. With Crook Point omitted, Point Arguello, a mainland site in central California, had the highest  $L_\infty$  and  $L_{max}$  values for this study. The age at  $L_\infty - 1$  for Point Arguello was estimated at 45 years (Table 5).

Only the 1988 survey period included data from both San Miguel Island and Point Arguello (Table 6). Comparisons for Point Arguello and the 3 locations on San Miguel Island were done for that period. The results of the likelihood ratio tests are listed in Table 4B. Differences occurred in the growth parameters among the 4 locations ( $p < 0.00001$ , common  $K$  and  $L_\infty$ ) (Table 4B).

To decide which differences were present, Point Arguello was compared with each island site for the 1988 period. Both Otter Harbor and Crook Point were similar to Point Arguello for that period ( $p = 0.15$  and  $p = 0.84$ , respectively, common  $K$  and  $L_\infty$ ) (Table 4B). Harris Point, however, was different from Point Arguello for that period ( $p < 0.00001$ , common  $K$  and  $L_\infty$ ) (Table 4B).

The year effect was previously shown to be absent for both Otter Harbor and Point Arguello. So, the combined yearly data for Otter Harbor was compared with the combined yearly data for Point Arguello. Otter Harbor represented a consistent island site, and Point Arguello represented a consistent mainland site. Using the combined data, the 2 sites showed different growth ( $p < 0.0001$ , common  $K$  and  $L_\infty$ ) (Table 4B).

This outcome, however, contrasted with the earlier result showing no difference between these 2 sites for the 1988 period only. The different outcomes may be attributed to 2 factors. The first factor was the sample size. The combined yearly data had 247 observations, which was more than 4 times larger than the 53 observations for the 1988 period. The growth parameters for the 2 sites were closer in the combined years than in the 1988 period (Table 5). The larger sample size for the combined years, however, made the test more sensitive to differences.

The other factor was that  $K$ , but not  $L_\infty$  contributed to the different outcomes. The test for  $K$  in the combined yearly data was statistically significant ( $p < 0.0001$ , common  $K$ ) (Table 4B), but not for  $L_\infty$  ( $p = 0.69$ , common  $L_\infty$ ) (Table 4B). The parameters for the combined data showed the difference. The  $L_\infty$  parameters were close: 133.5 for Otter Harbor, and 135.2 for Point Arguello. The  $K$  parameters were farther apart: 0.18 for Otter Harbor, and 0.11 for Point Arguello (Table 5).

## DISCUSSION

We feel comfortable with the growth estimates at Otter Harbor and Point Arguello. Both sites had growth parameters that were independent of time. The standard errors of the parameter estimates were small. In contrast, we had low confidence in the growth estimates at Harris Point and Crook Point. The growth parameters at Harris Point changed over time, which was an undesirable feature. At Crook Point, we were uncertain about the true values of the growth parameters, which was reflected in large standard errors of the parameter estimates.

An  $L_\infty$  smaller than the maximum observed size  $L_{max}$  was possible (Table 5). Although this is only a minor issue, we attempt an explanation. The  $L_\infty$  estimates the long term growth of an average abalone if it continues to grow according to the von Ber-

TABLE 4.

(A) Likelihood ratio tests comparing locations in a given year for black abalone at Otter Harbor, Harris Point, and Crook Point, San Miguel Island; (B) Likelihood ratio tests for the 1988–89 period comparing black abalone at Point Arguello with black abalone from Otter Harbor, Harris Point, and Crook Point, San Miguel Island. Likelihood ratio test comparing black abalone at Otter Harbor (all years) with black abalone at Point Arguello (all years).

A	n	Hypothesis	RSS	df	$\chi^2$	p-val
1986–87 Otter Harbor vs. Harris Point	239	Full parameters	815			
		Common $K$	1011	1	51.51	<.00001
		Common $L_\infty$	862	1	13.47	0.0002
1987–88 Otter Harbor vs. Harris Point	115	Common $K$ and $L_\infty$	1305	2	112.5	<.00001
		Full parameters	666			
		Common $K$	904	1	35.26	<.00001
1988–89 Otter Harbor vs. Harris Point vs. Crook Point	123	Common $L_\infty$	708	1	7.05	0.008
		Common $K$ and $L_\infty$	1352	2	81.46	<.00001
		Full parameters	1332			
1988–89 Otter Harbor vs. Harris Point vs. Crook Point	123	Common $K$	1485	2	13.36	0.001
		Common $L_\infty$	1383	2	4.63	0.10
		Common $K$ and $L_\infty$	1966	4	47.87	<.00001
B	n	Hypothesis	RSS	df	$\chi^2$	p-val
1988–89 Otter Harbor vs. Harris Point vs. Crook Point vs. Point Arguello	147	Full parameters	1446			0.002
		Common $K$	1599	3	14.81	<.002
		Common $L_\infty$	1508	3	6.23	0.01
		Common $K$ and $L_\infty$	2134	6	57.26	<.00001
1988–89 Otter Harbor vs. Point Arguello	53	Full parameters	433			
		Common $K$	465	1	3.79	0.051
		Common $L_\infty$	442	1	1.21	0.27
1988–89 Harris Point vs. Point Arguello	90	Common $K$ and $L_\infty$	465	2	3.8	0.15
		Full parameters	382			
		Common $K$	403	1	4.91	0.03
1988–89 Harris Point vs. Point Arguello	90	Common $L_\infty$	435	1	11.78	0.0006
		Common $K$ and $L_\infty$	494	2	23.18	<.00001
		Full parameters	859			
1988–89 Crook Point vs. Point Arguello	52	Common $K$	859	1	0.02	0.90
		Common $L_\infty$	859	1	0.04	0.84
		Common $K$ and $L_\infty$	864	2	0.35	0.84
1988–89 Otter Harbor (all years) vs. Point Arguello (all years)	247	Full parameters	1910			
		Common $K$	2034	1	15.45	<0.0001
		Common $L_\infty$	1911	1	0.16	0.69
		Common $K$ and $L_\infty$	2082	2	21.27	<.00001

The  $n$  is sample size,  $RSS$  is residual sum of squares,  $df$  is degrees of freedom,  $\chi^2$  is Chi-square value, and  $p$ -val is probability.

talanffy equation (Ricker 1975, p. 221). The  $L_\infty$  involves extrapolation, so that some caution in its interpretation is warranted (Knight 1968).

When interpreting the analysis, the sample size should be considered. We obtained different statistical outcomes for the comparison of Otter Harbor with Point Arguello when the sample size differed considerably. With a larger sample size, the test became more sensitive to smaller differences. Consideration should also be given to the fact that a difference may be statistically significant, but may not be biologically meaningful.

Three previous studies of black abalone growth were conducted in populations living in warm waters off southern California. Bergen (1975) at Santa Cruz Island found that growth slowed when black abalone were about 80 to 90 mm in length, which was a smaller size than what we found. While Bergen did not fit a growth model, her data suggested an  $L_\infty$  of about 125 mm.

Leighton and Boolootian (1963) conducted studies of black abalone at 2 coastal locations in southern California. At their sites,

black abalone grew about 22 to 32 mm during the first year. This was similar to the growth we found at Otter Harbor, but it was 2 to 6 times greater than at our other sites. They found that growth slowed when the abalone were about 130 mm, which was also similar to our findings.

Douros (1985) reported  $L_\infty$  values of 117 and 139, and  $K$  values of 0.37 and 0.47, respectively, at 2 sites on Santa Cruz Island. While his  $L_\infty$  parameters were similar to ours, his  $K$  parameters were greater than those for any other study.

Blecha et al. (1992) did tagging surveys at 4 sites near Diablo Canyon, in central California, about 66 km NNW of Point Arguello. These locations have cooler seawater, which was similar to Point Arguello and San Miguel Island. Thus, they are more appropriate for comparison with our estimates of growth. Von Bertalanffy and other values from that study are presented in Table 5.

For comparison among sites, Blecha et al. (1992) attributed growth variability, measured as a standardized difference, to exposure of the site to ocean waves. The standardized difference was

TABLE 5.

Von Bertalanffy growth parameters by location at San Miguel Island and Point Arguello; south and north Diablo Cove, Sea Haulout, and Postelsia Point, Diablo Canyon, data (Blecha et al. 1992) are included for comparison.

Location	Year	n	$L_{\infty}$	s.e.	K	s.e.	Age (yr) at		
							$L_{\infty} - 1$	$L_{\max}$	$(L_{\max} - L_{\infty})/s.e.$
Otter Harbor	1986-87	97	134.9	1.7	0.17	0.01		160	-
	1987-88	38	133.1	5.4	0.19	0.03		160	-
	1988-89	29	125.1	5.1	0.20	0.04		160	-
	All	164	133.5	1.8	0.18	0.01	27	160	14.7
Harris Point	1986-87	142	119.6	2.3	0.06	0.01	79	156	15.8
	1987-88	77	110.9	3.0	0.05	0.01	93	156	15.0
	1988-89	66	102.1	5.1	0.05	0.01	92	156	10.6
Crook Point	1987-88	6	-	-	-	-			-
	1988-89	28	138.7	39.1	0.12	0.09	41	142	-
Point Arguello	1988-89	24	133.1	4.9	0.11	0.03		170	-
	1989-90	59	136.3	4.7	0.11	0.01		170	-
	All	83	135.2	3.5	0.11	0.01	45	170	9.9
So. Diablo Cove	1979-84*		122.7	3.9	0.20		25	145	5.7
No. Diablo Cove	1979-84*		112.0	5.2	0.12		36	136	4.6
Seal Haulout	1979-84*		100.3	2.2	0.20		24	130	13.5
Postelsia Point	1979-84*		97.5	5.4	0.23		20	128	5.6

The n is the number of abalone,  $L_{\infty}$  is the mean asymptotic length (mm), K is the Brody growth coefficient, s.e. is the standard error,  $L_{\max}$  is the largest black abalone (mm) observed at a site, and \*is study period.

calculated as (maximum observed size  $L_{\max} - L_{\infty}$ )/standard error of  $L_{\infty}$ . By this measure, those sites where  $L_{\max}$  exceeded  $L_{\infty}$  by more than 10 standard deviations would be considered exposed to strong waves.

Both Otter Harbor and Harris Point fitted this growth variability criterion (Table 5). They are on the north end of San Miguel Island, where they experience heavy wave action. We do not have data, however, on long-term oceanographic conditions at these sites.

Various biological and physical factors not addressed in this study may explain differences in growth parameters among sites. For example, a lethal disease called withering syndrome (WS), which caused mass mortalities in black abalone populations, was discovered and eventually observed at every site, though sometimes not until after the study period. This disease had severe effects on growth studies at sites by killing the tagged abalone, and it compromised other work on black abalone. Withering syndrome

has a long (5 to 8 months) pre-patent period (the period between infection and noticeable symptoms) during which infected abalone would not grow well (pers. comm., Carolyn Friedman, Fish and Game Shellfish Disease Laboratory, Bodega Bay, CA).

At Crook Point, WS caused density declines of abalone (Fig. 3), and killed many tagged abalone, which lowered tag recovery. Without sufficient tag recoveries, growth could not be determined. At Harris Point, density declines were not apparent, but our results showed that growth declined from year to year (Figs. 2 and 3). The low K and declining  $L_{\infty}$  values, and the advanced age at  $L_{\infty} - 1$  showed growth inhibition.

Withering syndrome was unknown at both Otter Harbor and Point Arguello during the study period. At both locations, the growth parameters did not change during that time.

Of all the study sites, including Diablo Canyon, Point Arguello had the largest observed abalone. It was possibly a reflection of its remote location outside both the ranges of the predatory sea otter

TABLE 6.

Von Bertalanffy growth parameters for black abalone by year at San Miguel Island and Point Arguello.

Year	Location	n	$L_{\infty}$	s.e.	K	s.e.
1986-87	Otter Harbor	97	134.9	1.7	0.17	0.01
	Harris Point	142	119.6	2.3	0.06	0.01
1987-88	Otter Harbor	38	133.1	5.4	0.19	0.03
	Harris Point	77	110.9	3	0.05	0.01
	Crook Point	6	-	-	-	-
1988-89	Otter Harbor	29	125.1	5.1	0.2	0.04
	Harris Point	66	102.1	5.1	0.05	0.01
	Crook Point	28	138.7	39.1	0.12	0.09
	Point Arguello	24	133.1	4.9	0.11	0.03
1989-90	Point Arguello	59	136.3	4.7	0.11	0.01

The n is the number of abalone,  $L_{\infty}$  is the mean asymptotic length (mm), K is the Brody growth coefficient, and s.e. is standard error.

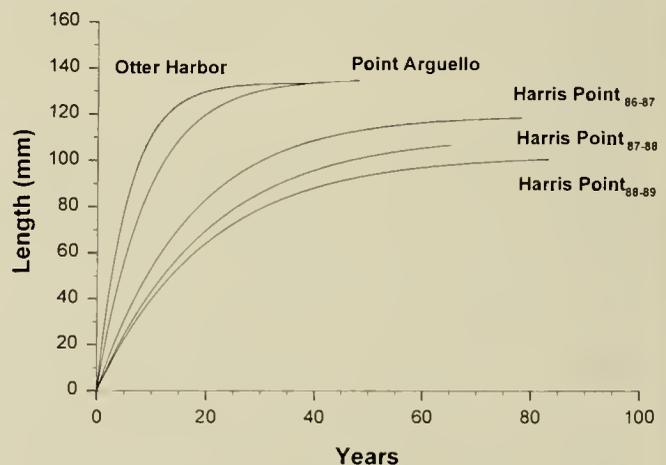


Figure 2. Black abalone growth curves at Otter Harbor, at Harris Point during 3 annual periods, and at Point Arguello.

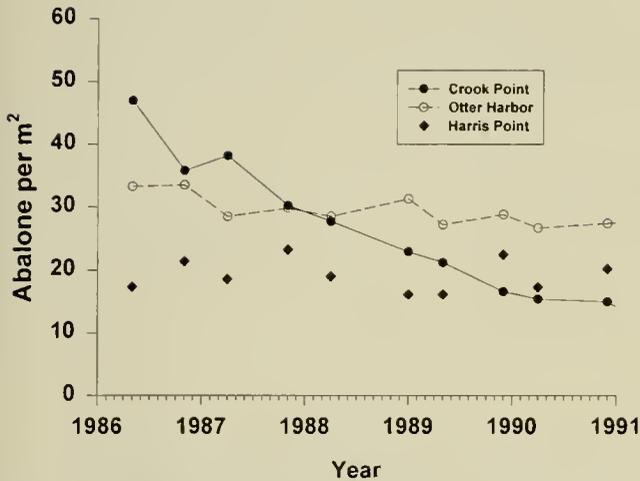


Figure 3. Density estimates of black abalone at Otter Harbor, Harris Point, and Crook Point on San Miguel Island for the years 1985 to 1990 (Channel Islands National Park Data).

and the commercial and recreational fisheries. Both sea otter predation and human harvest remove the largest abalone and thus depress  $L_{\infty}$ .

Of the sites we studied, the most consistent growth parameters were at Otter Harbor and Point Arguello. The growth at those 2 sites was a little faster than growth over a 5-year period at Diablo Canyon, which was farther north. Otter Harbor and Point Arguello

showed how black abalone could grow under favorable conditions. Thus, growth at these 2 sites suggested what could be the optimal rate of black abalone recovery from its current depressed condition.

Any recovery must presuppose that WS is no longer affecting stocks. This assumption is currently false, both at the islands and along the mainland. Resource recovery requires widespread black abalone recolonization, and the reestablishment of abalone with widely varying sizes in multiple cohorts. Such restoration is likely to take decades. The reestablishment of a viable fishery for black abalone will take even longer. Based on current fishery size limits, it would take decades for many black abalone to grow to commercially harvestable size.

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## BACTERIOLOGICAL STUDIES ON MUSSELS AND OYSTERS FROM SIX RIVER SYSTEMS IN PRINCE EDWARD ISLAND, CANADA

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**ABSTRACT** Eighty-five blue mussels and 46 eastern oysters were collected from shellfish growing areas of 6 river systems in Prince Edward Island, and examined for aerobic and anaerobic bacterial flora. Animals were collected in different seasons during a 14-month period. Of a total of 907 isolates recovered, 76% were aerobes, and 24% were anaerobes. Isolation rates of 572 aerobes grouped by genera were compared, with respect to sites of sampling and seasons. *Vibrio* was the most frequently isolated genus from animals originating from all river systems, except mussels collected from Cardigan River, which had *Staphylococcus* as the predominant flora. Mussels collected during winter yielded a higher percentage of *Staphylococcus* spp., while animals collected during other seasons had *Vibrio* as the major genus. Among 51 *Vibrio* isolates speciated with the aid of commercial identification systems, the most common species were *Vibrio* (*Listonella*) *anguillarum* from oysters, and *V. alginolyticus* and *V. splendidus* from mussels. *Vibrio parahaemolyticus* was isolated from 5% of animals. The most common *Staphylococcus* species was *S. capitis* subspecies *ureolyticus*. Among anaerobes *Clostridium perfringens* was the major species among identified isolates.

**KEY WORDS:** Mussels, oysters, bacterial flora, shellfish

### INTRODUCTION

Mollusc culture is a significant contributor to seafood production worldwide, and Prince Edward Island is the leader in mussel culture and oyster resource enhancement in Atlantic Canada. With the rapid development of aquaculture and technologies for intensive husbandry, bacterial diagnosis of shellfish diseases have become increasingly important. Although diseases due to bacteria in adult shellfish seem relatively insignificant compared to that for larval stages, mass mortalities of possible bacterial etiology have been observed in adult oysters as well as in juveniles in North America and Japan, especially during conditions of physiological stress such as spawning and high water temperatures (Sinderman 1990). There is considerable evidence to suggest that shellfish such as oysters maintain an indigenous microbial population (Souness and Fleet 1991). The association of microorganisms with animal tissues can be symbiotic or pathogenic or, as in the case of molluscs, coincidental as part of the filter feeding process. While certain bacteria such as *Vibrio anguillarum* and *V. alginolyticus* can cause disease and mortality in shellfish (Grishkowsky and Liston 1974, Elston 1989), others may make nutritional contributions to shellfish growth (Douillet and Langdon 1993), or may benefit shellfish in other ways such as in settlement of oysters due to *Shewanella colwelliana* (Tritar et al. 1992, Weiner et al. 1989). Typically, *Vibrio* species comprise commensal flora of shellfish, and some species may participate in the cycling of matter, i.e., decomposition of organic matter (West and Colwell 1984).

Although the generic composition of the natural bacterial flora of oysters was studied over 3 decades ago by Colwell and Liston (1960, 1962), no comprehensive investigation has been conducted in recent years. Furthermore, although there have been several studies on bacteria of public health significance, associated with shellfish, such as *Vibrio parahaemolyticus* and *V. vulnificus* (Ki-

iyukia et al. 1989, El-Sahn et al. 1982, Ruple and Cook 1992), no data is available on the natural bacterial flora of shellfish, particularly with reference to seasonal variation.

This paper presents the results of bacteriological analysis of cultured blue mussels (*Mytilus edulis*) and eastern oysters (*Crassostrea virginica*) from 6 river systems in Prince Edward Island. The main objectives of this study were to gain an overview of the various aerobic and anaerobic bacterial species found in oysters and mussels, and to understand possible variations in the flora with reference to seasons and sites.

### MATERIALS AND METHODS

Six river systems in Prince Edward Island (Fig. 1) where mussels and oysters are commercially grown were selected for this study. A total of 130 animals were collected between October 1991 and November 1992. Salinity in all sites was 20 to 25 ppt. Water temperatures during the summer months were in the order of 17 to 20°C. Fall water temperatures were 6 to 9°C in October and 2 to 5°C in November. River systems were frozen over during the winter months. Cultured blue mussels were sampled on 15 occasions, and a total of 85 animals were collected from 3 different sites: New London Bay, Cardigan River and Murray River. All 3 sites have been shown to be good mussel cultivation areas although high bacterial counts of the water threatened the closure of certain leases in Cardigan River from time to time (Bernard and Gallant 1992). There had been some problems with mussel die offs in New London Bay area in 1990, but the information gathered seems to point in the direction of inferior seed stocks as the possible cause (Bernard 1990). Oysters were sampled on 8 occasions, and a total of 45 animals were collected from Dunk River, East River and Lennox Island Channel. Samples were held on ice for transport to the laboratory. The shells were cleaned under running water with a stiff brush, and finally washed in sterile water. Mantles and digestive glands of all animals were collected aseptically, rinsed with sterile water, diluted 1:1 (W/V) with normal saline

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Figure 1. Map of Prince Edward Island showing sampling locations.

(0.85% NaCl), and blended separately in a stomacher (Lab-Blend 400 Stomacher, Seward Medical, London, U.K.) for 2 minutes. For cultivation of aerobic bacteria, tissue suspensions were plated onto blood agar (BA), BA with 2% NaCl, trypticase soy agar (TSA), TSA with 2% NaCl, MacConkey agar and thiosulfate-citrate-bile salt-sucrose agar (TCBS), and incubated at 20 and 37°C. For anaerobic bacteria, specimens were plated on pre-reduced BA and BA with 2% NaCl, and incubated in anaerobic jars or in an anaerobic chamber (Bactron-1, Anaerobe Systems, San Jose, CA) at 20 or 37°C. In addition, all samples were inoculated into cooked meat medium and incubated at 37°C. Isolates showing distinct colonial and microscopic morphology after Gram's staining, were subcultured and frozen at -70°C in 2% skim milk and/or brain heart infusion broth with 20% glycerol, pending identification. Anaerobic isolates were checked for absence of aerobic growth before storage.

The aerobic isolates were divided into Gram-negative and Gram-positive bacteria. Based on several different tests, Gram-negative bacteria were grouped into *Vibrio*-like, *Aeromonas* or *Pseudomonas*-like, Enterobacteriaceae, and Gram-negative bacteria which did not fall into any of the above-mentioned groups. The tests included oxidase, sensitivity to vibriostat 0/129, growth on TCBS, fermentation of glucose and lactose, nitrate reduction, citrate utilization, urease and indole production, catalase production and reaction on triple sugar iron agar. Details of these tests and the criteria for grouping are described elsewhere (Smibert and Krieg 1981, Collins et al. 1989, Hendrickson and Krenz 1991). Salt tolerance and production of arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase were tested for the classification of vibrios as outlined by Kelly et al. (1991). Gram-positive bacteria were grouped on the basis of morphology, and several of the tests listed previously. Fermentation of salicin, inositol, mannitol, maltose, arabinose, sucrose and trehalose were also tested. The data were tabulated, analyzed and sorted into groups of similar isolates using a computer software program Quattro Pro version 4.0. Isolates from each group capable of growing at 30°C were tested with the Biolog MicroStation™ System, Release 3.01 (Bi-

olog, Inc., Hayward, CA). Biolog 96 well microtitre plates, pre-loaded with metabolic tests for Gram-negative and Gram-positive bacteria, were inoculated, incubated at 30°C, and the results read as per the manufacturer's directions. After a 4 hr incubation period, only an "excellent identification" (similarity index  $\geq 0.75$ ) was considered acceptable. After incubation for 24 hr a similarity index of  $\geq 0.5$  were required for an acceptable genus and species identification. These criteria of the Biolog identification system have been found satisfactory by other workers (Miller and Rhoden 1991). The identification of *Vibrio* isolates belonging to human pathogenic species were confirmed by MicroScan® identification system (Baxter Diagnostics Inc., Deerfield, IL). Isolates identified as *Vibrio cholerae* were tested by slide agglutination using OI polyvalent antiserum (Difco Laboratories, Detroit, MI).

Anaerobic bacteria were grouped on the basis of cultural characteristics, cell morphology, hemolytic reactions, sensitivity to AN-IDENT discs (Unipath, Nepean, Ontario), and presumptively identified following the methods described by Engelkirk et al. (1992). Species confirmation was done using RapID ANA II system (Innovative Diagnostic Systems, Inc., Atlanta, GA) and/or AN-IDENT (Analytical Products, Mainview, NY) identification systems.

## RESULTS

Of a total of 907 bacterial isolates recovered from mussels and oysters 689 (76%) were aerobes and 218 (24%) were anaerobes. Most of the isolates (80%) were cultured from digestive glands, the percentage for aerobes and anaerobes were 68% and 91% respectively. The rates of isolation of aerobes from the digestive glands of mussels and oysters were 75% and 61% respectively. Sixty-four percent of the aerobes were recovered from mussels at incubation temperatures of 20°C, and 36% at 37°C, whereas only 46% of the aerobes from oysters were cultured at 20°C, the remaining 54% were isolated at 37°C.

Of the 689 aerobic isolates, 572 were grouped into 8 genera. Sixty-eight percent of 572 isolates were Gram-negative bacteria. Table 1 shows the distribution of these generic groups from mussels and oysters collected from different sites. Thirty-four percent of the isolates belonged to the genus *Vibrio*, which was the predominant genus in animals from all sites, except mussels collected from Cardigan River. The predominant genus isolated from mussels from Cardigan River was *Staphylococcus*, comprising 28% of the isolates. In general, *Pseudomonas/Shewanella* were the second major group. Enterobacteriaceae consisted of only 4% of all isolates. Table 2 shows seasonal distribution of aerobic bacterial isolates from mussels and oysters. While the predominant group in fall and summer was *Vibrio*, in winter 35% of all isolates from mussels were *Staphylococcus* spp., and only 12% belonged to *Vibrio* spp. The *Pseudomonas/Shewanella* group, as well as *Bacillus* and *Staphylococcus* spp., exceeded *Vibrio* species in mussels sampled during winter.

Of 193 *Vibrio* isolates, only 51 could be speciated using Biolog MicroStation™ system; due mainly to the lack of growth of most isolates at 30°C (the incubation temperature used by the Biolog system). The predominant species in mussels were *Vibrio splendidus* and *V. alginolyticus*, with 7.1% of animals harboring each of these bacteria (Table 3). Among oysters, 8.9% of animals yielded *V. anguillarum*. *V. parahaemolyticus* and *V. vulnificus* were isolated from 6.7% of oysters. These species were less frequently isolated from mussels. All isolates of *V. parahaemolyti-*

TABLE 1.  
Isolation of groups of aerobic bacteria from various sites.

Bacteria	Mussels			Oysters			Total No. (%)
	New London Bay n = 21	Cardigan River n = 41	Murray River n = 23	East River n = 24	Lennox Island n = 18	Dunk River n = 3	
	No. (%)*	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
<i>Vibrio</i> spp	38 (37)	55 (25)	47 (40)	37 (44)	12 (32)	4 (50)	193 (34)
<i>Aeromonas</i> spp	12 (12)	10 (5)	19 (16)	3 (4)	6 (16)	0 (0)	50 (9)
<i>Pseudomonas</i> / <i>Shewanella</i>	19 (19)	57 (23)	27 (23)	18 (21)	3 (8)	40 (50)	122 (21)
<i>Enterobacteriaceae</i>	1 (1)	5 (2)	5 (4)	10 (12)	2 (5)	0 (0)	23 (4)
<i>Staphylococcus</i> spp	17 (17)	63 (28)	12 (10)	9 (11)	9 (24)	0	110 (19)
<i>Bacillus</i> spp	14 (14)	35 (16)	7 (6)	8 (9)	5 (14)	0	69 (12)
<i>Streptococcus</i> spp	1 (1)	3 (1)	1 (1)	0	0	0	5 (1)
Total no.	102	222	118	85	37	8	572

\* Percentages relate to total number for individual river system.

*cus*, *V. vulnificus* and *V. cholerae* (non-01) were isolated from animals collected during summer and fall, and not from those collected during winter (data not shown). All *V. vulnificus* isolates were indole negative, and belonged to biotype 2. The second largest group of bacteria was *Pseudomonas/Shewanella*. In the *Pseudomonas/Shewanella* group, 11 isolates were speciated by Biolog system. Six isolates were identified as *Pseudomonas fluorescens*, 4 from mussels and one from an oyster. Three isolates were identified as *Shewanella putrefaciens*. Among the *Aeromonas* isolates, 4 were identified as *A. hydrophila*, 3 from oysters and one from a mussel. Seventy-five percent of the identified Enterobacteriaceae isolates belonged to the genera *Providencia* and *Serratia*, with *Providencia alkalicifaciens* and *Serratia fonticola* species predominating. One isolate from a mussel was identified as *Butiauxella agrestis*.

Of the 110 *Staphylococcus* isolates, 46 were speciated (Table 4). *Staphylococcus capitis* subspecies *ureolyticus* was the predominant species in both mussels and oysters, with 20% of staphylococcal isolates belonging to this species. Twelve percent of isolates from mussels were *S. hominis*.

Of 69 *Bacillus* isolates, 26 (38%) were identifiable by Biolog system. These consisted of *B. pumilus*, which represented 10% of all *Bacillus* isolates, *B. thuringiensis* (8.7%), *B. subtilis*, var. *globigii* (7.2%), and *B. polymyxa*, *B. mycoides*, *B. cereus*, *B. megaterium* and *B. sphaericus*, each less than 3% of total isolates. Of a total of 5 streptococcal isolates, 2 were identified as *S. mitis*, and one as *S. gordonii*, all three from mussels.

Of 109 aerobic isolates not belonging to the previously mentioned bacterial groups, 9 were *Acinetobacter johnsonii*, one from a mussel and 8 from oysters. Two were *Flavobacterium indologenes* from oysters. *Micrococcus naucinus* was isolated from 7 mussels and 1 oyster. Other identified isolates consisted of two each of *Corynebacterium pseudodiphtheriticum*, *C. jeikeium*, and *C. ureolyticum*, all from mussels, 1 isolate of *C. xerosis* from an oyster and 2 isolates of *Rothia dentrocaricosa*, one from a mussel and the other from an oyster.

Of 218 anaerobic isolates, 160 were from mussels and 58 were from oysters. Seventy-two percent of total anaerobic isolates were Gram-positive bacteria. Only 46 isolates could be identified to species level. *Clostridium perfringens* comprised 24% of identi-

TABLE 2.  
Seasonal variation in groups of aerobic bacteria isolated from mussels and oysters.

Bacteria	Mussels				Oysters			Total No. (%)
	Fall '91 n = 13	Fall '92 n = 36	Winter '92 n = 18	Summer '92 n = 18	Fall '91 n = 9	Fall '92 n = 18	Summer '92 n = 18	
	No. (%)*	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
<i>Vibrio</i> spp	45 (46)	56 (41)	13 (12)	26 (26)	7 (41)	10 (37)	36 (42)	193 (34)
<i>Aeromonas</i> spp	6 (16)	19 (14)	2 (2)	14 (14)	0 (0)	1 (4)	8 (9)	50 (9)
<i>Pseudomonas</i> / <i>Shewanella</i>	42 (43)	15 (11)	32 (29)	8 (8)	6 (35)	6 (22)	13 (15)	122 (21)
<i>Enterobacteriaceae</i>	2 (2)	4 (3)	3 (3)	3 (3)	4 (24)	2 (7)	6 (7)	23 (4)
<i>Staphylococcus</i> spp	2 (2)	24 (18)	38 (35)	28 (28)	0 (0)	8 (30)	10 (12)	110 (19)
<i>Bacillus</i> spp	0	17 (13)	20 (18)	19 (19)	0 (0)	0 (0)	13 (15)	69 (12)
<i>Streptococcus</i> spp	1 (1)	1 (1)	2 (2)	1 (1)	0 (0)	0 (0)	0 (0)	5 (1)
Total no.	98	136	110	99	17	27	86	572

\* Percentages relate to total number for individual season.

TABLE 3.  
Species of *Vibrio* from mussels and oysters.

Species	Total Number	Number Positive (%)	
		Mussels n = 85	Oysters n = 45
<i>V. anguillarum</i> ( <i>Listonella</i> )	7	3 (3.5)	4 (8.9)
<i>V. splendidus</i>	7	6 (7.1)	1 (2.2)
<i>V. parahaemolyticus</i>	7	4 (4.7)	3 (6.7)
<i>V. alginolyticus</i>	6	6 (7.1)	0
<i>V. pelagius</i>	6	4 (4.7)	2 (4.4)
<i>V. vulnificus</i> (biotype-2)	5	2 (2.4)	3 (6.7)
<i>V. damsela</i> ( <i>Photobacterium</i> )	3	1 (1.2)	2 (4.4)
<i>V. mediterranei</i>	2	1 (1.2)	1 (2.2)
<i>V. carchariae</i>	2	2 (2.4)	0
<i>V. cholerae</i> (Non-01)	2	2 (2.4)	0
<i>V. metschnikovii</i>	1	1 (1.2)	0
<i>V. harveyi</i>	1	0	1 (2.2)
<i>V. tubiashii</i>	1	1 (1.2)	0
<i>V. ordalii</i>	1	1 (1.2)	0
Total	51	34	17

fied isolates. Fourteen percent of mussels and 2% of oysters were positive for *C. perfringens*. This organism was isolated in both fall (80%) and winter (20%). The percentages of other species among the identified anaerobic isolates were *Clostridium difficile*, 15%; *C. sporogenes*, 13%; *Bacteroides buccae*, 8.7%; *Fusobacterium mortiferum*, 8.7%; and *C. bifermentans*, 6.5%. Other species represented less than 5% of the identified anaerobes.

#### DISCUSSION

The results of the present study demonstrate that a variety of bacteria, both aerobic and anaerobic are present in the internal organs of healthy mussels and oysters, irrespective of the source and season. The predominant flora of oysters have been reported as of *Pseudomonas*, *Vibrio*, *Aeromonas*, *Acinetobacter* and *Flavobacterium* (Colwell and Liston 1960, Vasconcelos and Lee 1972). Our results are in agreement with these earlier observations with regard to *Vibrio* and *Pseudomonas*, and 55% of the aerobic

TABLE 4.  
Species of *Staphylococci* from mussels and oysters.

<i>Staphylococcus</i> Species	Total Number	Mussels n = 85	Oysters n = 45
<i>S. capitis</i> ss <i>ureolyticus</i>	22 (20) <sup>1</sup>	18 (20)	4 (22)
<i>S. hominis</i>	12	11	1
<i>S. epidermidis</i>	7	5	2
<i>S. cohnii</i>	1	0	1
<i>S. warneri</i>	1	1	0
<i>S. saprophyticus</i>	1	1	0
<i>S. caprae</i>	1	1	0
<i>S. xylosus</i>	1	1	0
Other <i>Staphylococci</i> spp.	64	54	10
Total	110	92	18

<sup>1</sup> Percentage of *S. capitis* ss *ureolyticus* to total number of *Staphylococcus* isolates.

isolates were Gram-negative bacteria in our study. It was also suggested by Colwell and Liston (1960) that oysters maintain an indigenous gut flora, retaining some bacteria and eliminating others. The majority of our isolates were recovered from the digestive glands in case of both mussels and oysters. One interesting difference in our results was with respect to mussels sampled in winter, in which Gram-positive bacteria tend to predominate. Furthermore, in mussels from Cardigan River, the number of staphylococci isolated was higher than that of *Vibrio*.

In the present study, the predominant *Vibrio* species identified were *V. alginolyticus* and *V. splendidus* from mussels and *V. (Listonella) anguillarum* from oysters. It has been shown over 2 decades ago that vibrios resembling *V. anguillarum* and *V. alginolyticus* can be isolated from overtly healthy, diseased or moribund bivalve molluscs and from their environments in highly productive commercial shell-fishing areas (Lovelace et al. 1968, Tubiash et al. 1970).

All isolates of the 3 major human pathogenic vibrios, *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* were isolated in fall and summer only. Similar observations have been made by other workers with regard to oysters. *Vibrio vulnificus* was not detected in winter in either sea water or oyster samples by Tamplin et al. (1982) and seasonal variations in isolation from shellfish samples and their environment were also observed (O'Neill et al. 1992, Ruple and Cook 1992). Alonzo et al. (1981) reported the increased occurrence of *V. parahaemolyticus* in mussels cultivated in marine environments in Italy, and attributed the increase to organic waste matter, microalgae and zooplankton in summer. Seasonal distribution of *V. parahaemolyticus* and its isolation only in warmer months only from marine environments and shellfish was also reported from Japan (Kiiyukia et al. 1989) and from Egypt (El-Sahn et al. 1982). *Vibrio* species of public health significance, particularly *V. vulnificus*, *V. alginolyticus* and *V. cholerae*, were found in mussels from Nova Scotia coastal waters (Badley et al. 1990). These species were also isolated from mussels in the present study. *Vibrio metschnikovii*, a potential human pathogen, was isolated from one mussel in our study. This organism has been found only in a small percentage of mussels in other studies (Badley et al. 1990, Buck 1991). *Vibrio damsela*, currently classified as *Photobacterium damsela* (Austin and Austin 1993), a marine bacterium that has been exclusively associated with human wound infections (Janda et al. 1988) was isolated from 3 animals in the present study. In a study on *Vibrio* species in mussels and sea water at a shellfish hatchery located in Spain, Ortigosa et al. (1989) found that *V. alginolyticus* and *V. harveyi* were the most frequently isolated species. Other species isolated in their study included *V. pelagius*, *V. damsela*, *V. splendidus*, *V. parahaemolyticus*, *V. anguillarum*, *V. mediterranei*, *V. tubiashii*, and *V. cholerae*. These species were also found in our study.

*Vibrio* and *Pseudomonas* have been reported as dominating the bacterial flora of marine shellfish from unpolluted regions, whereas *Lactobacillus* and *A. hydrophila* are more common in oysters from regions where faecal contamination is a problem (Colwell and Liston 1960, Sakata 1989). The predominant bacterial flora in mussels and oysters in our study were *Vibrio* and *Pseudomonas*. The isolation of *Aeromonas* was low and organisms such as *Lactobacillus*, *E. coli* or *Klebsiella* were not isolated. Enteric bacteria such as *E. coli* and fecal coliforms are usually effectively purged from shellfish in clean water (Jones et al. 1991). Indigenous bacteria may remain, since shellfish such as oysters seem to have a selective mechanism by which they seques-

ter and maintain some microbial species in a recoverable state, but not other species (Souness and Fleet 1991).

Cardigan River has a problem of high coliform counts in the water (Bernard and Gallant 1992). However, only 2% of major groups of bacteria isolated from the mussels from Cardigan River represented Enterobacteriaceae. In this context, it may be noteworthy that the fecal coliform content of water and shellfish may not always be correlated (Valiela et al. 1991).

Other Gram-negative bacteria identified in our study were *Acinetobacter* and *Flavobacterium*. Brown et al. (1988) reported that Gram-negative rods, particularly the genera *Flavobacterium*, *Achromobacter* (*Acinetobacter*), *Pseudomonas* and *Vibrio* predominated in water columns above oyster beds. There was no indication of the flora of the oysters themselves.

Staphylococci and *Bacillus* spp. were the most common Gram-positive isolates in the present study. According to Lauckner (1983), the Gram-positive flora of bivalves include mainly *Bacillus*, *Corynebacterium* and *Micrococcus*. Our results have shown that corynebacteria and micrococci are much less common compared to *Staphylococcus* and *Bacillus* spp. in mussels and oysters from the P.E.I. river systems studied.

The majority of our anaerobic isolates were Gram-positive bacteria, with *C. perfringens* predominating among isolates identified to species level. *C. perfringens* is common in soil and water. Shellfish can get contaminated due to rain run off into shellfish growing areas. Problems with rain run off in P.E.I. occur mainly in the spring. Unfortunately, sample collections did not commence

until late July. A study by Madden et al. (1986) showed that *C. perfringens* persists longer than *E. coli* in sea water and shellfish. Animal and human excrement contain *C. perfringens* and either of these can contaminate soil, particularly during rainy seasons. *C. perfringens* is considered an indicator of faecal pollution. This organism was isolated from 12% of mussels from Cardigan River.

In conclusion, this study shows that mussels and oysters from 6 river systems in Prince Edward Island have *Vibrio* species as the predominant bacterial flora, except the mussels collected from Cardigan River area and those collected during winter. In these samples, staphylococci tend to be the major group. Human pathogenic vibrios were present in a small proportion of samples. The percentages of mussels yielding these organisms, with the exception of *V. parahaemolyticus*, were much less than that found in another study in Atlantic Canada (Badley et al. 1990). Enterobacteriaceae constituted a definite minority in all samples, and coliforms were not found in mussels or oysters using the isolation methods in this study.

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## TECHNIQUES FOR THE RECOVERY OF *BACTEROIDES VULGATUS* FROM SHELLFISH

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**ABSTRACT** Clinical techniques and prereduced anaerobically sterilized (PRAS) media were utilized for the isolation and identification of *Bacteroides vulgatus* from shellfish. *B. vulgatus* is one of the most commonly recovered anaerobic bacteria from the human colon and outnumbers fecal coliforms in the human intestine a thousandfold. Identification of *B. vulgatus* was verified by the organism's resistance to kanamycin, vancomycin, colistin, ability to grow in the presence of bile, and inability to hydrolyze esculin. Sixty-six shellfish samples were examined. Twenty-eight of these samples (42%) possessed levels of fecal coliforms exceeding the established wholesale market limit. Fifteen (23%) tested positive for the presence of *B. vulgatus*. Of these 15 *B. vulgatus* positive samples, eight (53%) had fecal coliform levels greater than the wholesale market standard, five (33%) had fecal coliform levels less than the market standard, and two (13%) had nondetectable fecal coliform levels. An experimental PRAS medium, *Bacteroides vulgatus* Selective agar, was developed and tested for a more rapid recovery and identification of *B. vulgatus* from shellfish.

**KEY WORDS:** Shellfish sanitation, *Bacteroides*, indicator organism, PRAS media

### INTRODUCTION

Bivalve shellfish (e.g., oysters, mussels, and clams) are popular food items and are frequently eaten raw or partially cooked. This mode of consumption coupled with the filter-feeding habit of these shellfish makes these molluscs important vehicles of disease transmission. Between 1900 and 1992, 661 outbreaks of shellfish-associated enteric illness involving 14,365 cases had been reported nationally (Rippey 1992). The majority of these cases arose from fecally contaminated shellfish.

Fecal coliforms are the current indicators of the sanitary quality of shellfish with the molluscan shellfish wholesale market standard being less than or equal to 230 most probable number (MPN) fecal coliforms per 100 grams of shellfish meat. The coliform standard was implemented in 1925 in response to the 1924 outbreak of typhoid fever and has resulted in the decrease of shellfish associated typhoid fever (USFDA 1992). Since 1954 there have not been any cases of shellfish associated typhoid fever reported in the US (Rippey 1992). However, reports of outbreaks associated with sewage-contaminated shellfish, involving agents such as hepatitis A (CDC 1990), Norwalk virus (Guzewich and Morse 1986, Morse et al. 1986), *Shigella* spp., other enteric bacteria (Earampamoorthy and Koff 1975, Rippey 1992), and outbreaks of unknown etiology (Rippey 1992), have not decreased. The efficacy of the coliform group as indicators of fecal contamination has thus become the focus of much scrutiny and other potential indicators are being sought.

*B. vulgatus* is a member of the *Bacteroides fragilis* group and is one of the most prevalent organisms in the human lower intestine outnumbering coliforms a thousand to one and accounting for approximately 20-30% of all colon isolates (Fiksdal et al. 1985). It is an anaerobic organism and does not occur naturally in the environment nor does it persist for extended periods of time in the environment as do some members of the coliform group (Fiksdal et al. 1985). Clinical methods have defined specific characteristics and media that enable the isolation and identification of the *B.*

*fragilis* group. *B. vulgatus* is the only member of the *B. fragilis* group that does not typically hydrolyze esculin (Summanen et al. 1993), thus allowing a rapid differentiation of *B. vulgatus* from other members of the *B. fragilis* group.

The purpose of this investigation was to determine whether *B. vulgatus* could be isolated from shellfish using prereduced anaerobically sterilized (PRAS) method. PRAS media is recognized as superior to non-PRAS media in the isolation of anaerobic bacteria in clinical settings (Summanen et al. 1993). An experimental PRAS medium, *Bacteroides vulgatus* Selective Agar (BVA) was developed to expedite the isolation and identification of *B. vulgatus* from shellfish.

### MATERIALS AND METHODS

This investigation was designed as a preliminary study to determine whether *B. vulgatus* could be recovered from shellfish by employing clinical methods utilizing PRAS to isolate and differentiate members of the *B. fragilis* group. PRAS media has higher recovery rates with respect to anaerobic bacteria than non-PRAS media (Summanen et al. 1993). The presumptive identification of *B. vulgatus* was based on criteria established by Summanen et al. (1993) including: 1) circular edged, grayish, convex, translucent colonial morphology, 1-3 mm in diameter; 2) gram negative rod cellular morphology; 3) antibiotic resistance to kanamycin, vancomycin, and colistin; 4) growth on *Bacteroides* Bile Esculin agar (BBE) with no esculin hydrolysis; 5) growth on laked-blood vancomycin agar (LKV); and, 6) indole negative reaction.

#### *Shellfish Samples and Preparation*

A total of 66 shellfish samples, [46 *Crassostrea* spp. (oyster) samples, 19 *Mytilus* spp. (mussel) samples, and one *Clinocardium* spp. (cockle) sample] were collected at specific monitoring sites along the coast of California. These locations ranged from pristine coastal areas with no human sewage outfall, to conditionally approved, and restricted growing areas (impacted by fecal contamination, especially after rainfall) as defined by California and the National Shellfish Sanitation Program Manual of Operations (1992). The samples were processed according to shellfish sanitation protocols (American Public Health Association 1970). Du-

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plicate samples were obtained from each shellfish sample set. One of the duplicates was analyzed for total and fecal coliforms using most probable number (MPN) analysis, and for heterotrophic bacteria using heterotrophic plate count (HPC) enumeration. The other sample was processed following the same recommended procedures only under anaerobic conditions (90% N<sub>2</sub>, 5% CO<sub>2</sub>, 5% H<sub>2</sub>) in a Bactron II anaerobic chamber (Anaerobe Systems, Inc., San Jose, CA) for the detection of *B. vulgatus*.

Two hundred grams of meat and associated liquors were shucked into a sterile stainless steel blender jar per sample. The contents were then blended for 120 seconds, aerobically. One hundred grams was removed from the blender jar and stored at 4°C in a sealed, sterile 8 ounce polypropylene jar for later (within 24 hr) processing and analysis under anaerobic conditions. The other subsample was processed for total and fecal coliform MPN analysis and HPC analysis in accordance with standard methods (American Public Health Association 1970).

#### Anaerobic Sample Analysis

All post-blender manipulations were carried out under anaerobic conditions within the chamber. Two dilutions (1/10 and 1/100) of the blended sample were inoculated using the spread plate method, in duplicate, onto different prereduced anaerobically sterilized PRAS media for primary isolation: Brucella blood agar (BBA) and phenylethylalcohol blood agar (PEA) (Anaerobe Systems, Inc., San Jose, CA). BBA, a non-selective medium, was utilized to yield an overall count of both obligate and facultative anaerobes in the shellfish samples examined. PEA inhibits the growth of facultative anaerobes, including members of the coliform group, while allowing all obligate anaerobes to grow.

The inoculated BBA and PEA medium were incubated anaerobically for 48 hours at 35°C, after which colonies on each plate were counted. One of each colonial morphotype that was 1–3 mm in diameter, a circular entire, convex, grayish, and translucent colony (characteristic of the *B. fragilis* group), was subcultured for 24 hours at 35°C onto secondary PRAS media: BBE agar, laked-blood vancomycin agar (LKV) (Anaerobe Systems, Inc., San Jose, CA), and BBA with 3 antibiotic differentiation disks [kanamycin (1000 µg), colistin (10 µg), and vancomycin (5 µg) (Difco, Detroit, MI)]. BBE agar is highly selective for members of the *B. fragilis* group and affords rapid differentiation of *B. vulgatus* from other members of the *B. fragilis* group based on growth with no hydrolysis of esculin. Resistance to the 3 antibiotics is indicative of *B. fragilis* group members, as is the ability to grow on LKV agar.

Cultures demonstrating: 1) resistance to each of the 3 antibiotic disks (<10 mm inhibition zone around each disk); 2) growth on LKV agar; and, 3) growth on BBE agar (regardless of esculin reaction) were identified as *B. fragilis* group organisms. Indole reactions of isolates meeting the above 3 criteria were then used to speculate these organisms within the *B. fragilis* group; indole negative organisms being either *B. vulgatus*, *B. distasonis*, or *B. fragilis*. Indole negative and esculin negative isolates meeting the above criteria were classified presumptively as *B. vulgatus* organisms.

#### *Bacteroides vulgatus* Selective Agar (BVA)

An experimental medium, prepared according to PRAS conventions, combining the ingredients of BBE agar and the 3 antibiotics kanamycin (200 mg/L), vancomycin (7.7 mg/L), and colistin (189 units/L) was formulated to expedite the isolation and differentiation of *B. vulgatus* from the other members of the *B.*

*fragilis* group. This medium was labeled as BVA. BVA was tested, under anaerobic conditions, for its ability to grow the facultatively anaerobic bacteria *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Proteus mirabilis*, and the obligately anaerobic bacteria *B. vulgatus*, *B. fragilis*, *Fusobacterium necrophorum*, *F. nucleatum*, *Peptostreptococcus anaerobius*, *Clostridium perfringens*, and *C. difficile*. BVA inhibited the growth of all the above bacteria except for *Bacteroides vulgatus* and *B. fragilis*, which were recovered within 24 hours (Anaerobe Systems, Inc. 1992). Based on these results, organisms able to grow on this medium were considered presumptive *B. fragilis* group members, with those that did not hydrolyze esculin presumptively identified as *B. vulgatus*.

Some of the samples were tested using BVA as an additional primary plating medium, following the same procedures outlined above for the BBA and PEA media. One mussel homogenate was spiked with  $7.5 \times 10^8$  *B. vulgatus* organisms to test the recovery of the organism on BVA as well as on BBA and PEA.

## RESULTS

Of the 66 shellfish samples examined, 42% (28 total, 20 oyster samples and 8 mussel samples) resulted in fecal coliform levels exceeding the acceptable level of 230 MPN/100 g shellfish, while 23% (15 total, 11 oyster samples and 4 mussel samples) tested positive for the presence of *B. vulgatus* (Fig. 1). Of these 15 *B. vulgatus* positive samples, eight (6 oyster samples and 2 mussel samples) had fecal coliform levels greater than the wholesale market standard of 230 MPN/100 g shellfish, five (4 oyster samples and 1 mussel sample) had fecal coliform levels less than the market standard, and two (1 oyster sample and 1 mussel sample) had nondetectable levels of fecal coliforms.

A ranks sign test performed on the number of *B. vulgatus* positive samples versus the number of samples exceeding the 230 MPN/100 g fecal coliform value, showed that the presence of *B. vulgatus* in the shellfish samples was not related to the fecal coliform density ( $p < 0.05$ ).

The spiked sample yielded uniform *B. vulgatus* colonies on the experimental BVA. This same spiked sample yielded various col-

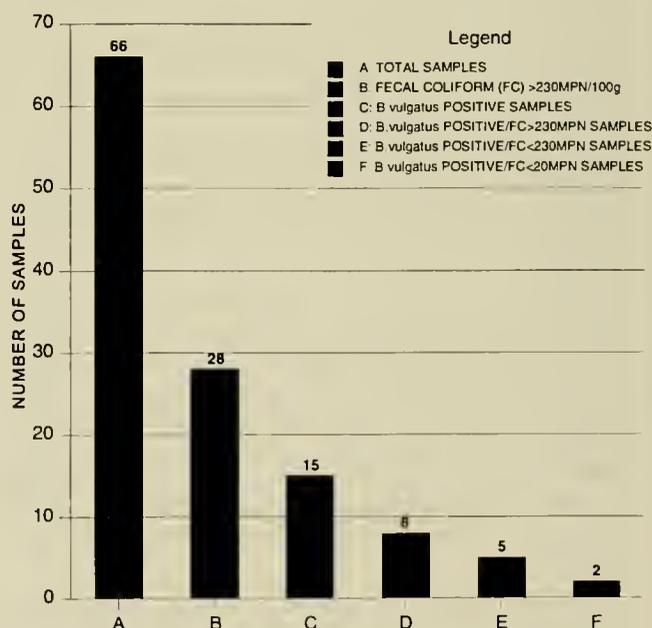


Figure 1.

ony types, including *B. vulgatus*, on the non-selective BBA medium as well as on the more selective PEA medium. BVA's recovery of *B. vulgatus* was calculated at 0.01%.

#### DISCUSSION

The ability to recover *B. vulgatus* from shellfish was demonstrated. Fifteen (23%) of the 66 shellfish samples analyzed were found to contain *B. vulgatus*. Seven of the 15 samples showed various densities of fecal coliforms, all below the 230 MPN/100 g limit. The exact significance of this statistic is unknown, but it does point out the statistical dependence upon which the current market standard is based. The fecal coliforms consist of organisms not always associated with fecal contamination (Greenberg et al. 1992), whereas *B. vulgatus* has been shown to be one of the most dominant bacteria in the intestines of humans (Hentges 1989), and since it is an obligate anaerobe it should not exist in the environment for more than a few days. Given this information, the detection of *B. vulgatus* in shellfish seems to be a more reliable indication of fecal contamination than does a fecal coliform MPN value of greater than 230 MPN/100 g shellfish.

*B. vulgatus* was also detected in 2 samples whose fecal coliforms densities were nondetectable (<20 MPN/100 g shellfish). One explanation for this result may be that since *B. vulgatus* outnumbers the fecal coliforms in the human intestine a thousandfold, then one is more likely to recover *B. vulgatus* than fecal coliforms especially in the cases of recent fecal contamination. Another possible explanation may be differential rates in reduction of recoverability. Power and Collins (1989) have shown that differential recoverability rates for *E. coli* and viruses occur in mussels; this phenomenon may explain the disparate result between the 2 aforementioned samples, i.e., the presence of *B. vulgatus* and absence of fecal coliforms.

There are limitations in the use of *B. vulgatus* as an indicator organism. Allsop and Stickler (1984) have demonstrated that the *B. fragilis* group members die off more rapidly than *E. coli* in both fresh and ocean waters. However, considering that *E. coli* has been shown to persist in the sediments of shellfish growing waters

(Gerba and Mcleod 1976), the detection of *B. fragilis* group members specifically *B. vulgatus*, may be a more reliable indication of recent fecal contamination than the fecal coliforms and *E. coli*, particularly when shellfish and shellfish growing waters are examined after recent rainfall.

The recovery of *B. vulgatus* from the spiked sample demonstrates BVA's ability to isolate *B. vulgatus* from shellfish. That the spiked samples yielded only 1 type of colony (that of *B. vulgatus*) on the BVA medium compared to several colony types yielded on BBA and PEA (*B. vulgatus* and other nonidentified bacteria) also demonstrates BVA's selective ability over BBA and PEA. The relatively low recovery rate of 0.01% reflects the need for replicate spike-recovery analyses to be performed. The recovery rate of 0.01% may appear low, but the average *B. vulgatus* content in dry weight of feces is about  $10^{11}$  organisms per gram (Hentges 1989), still yielding a recovery of about  $10^9$  organisms per gram. Further investigation into the enumeration of *B. vulgatus* in shellfish growing waters and in shellfish using BVA is warranted.

The potential of *B. vulgatus* as an indicator of fecal contamination is promising. *B. vulgatus* does not occur naturally in the environment; *B. vulgatus* is one of the most abundant organisms in the lower intestines of humans; and its isolation and identification in shellfish has been demonstrated by this study. BVA may be suitable as a rapid (within 24–48 hours) detection medium for *B. vulgatus* from shellfish and shellfish growing waters. Ongoing work is being conducted to test this medium in membrane filtration studies with shellfish growing waters to further examine its feasibility as a medium for the routine monitoring of water quality.

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**PROCEEDINGS OF THE SPECIAL SYMPOSIUM:  
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## AN OVERVIEW OF THE STATUS OF FRESHWATER CRAWFISH CULTURE

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**ABSTRACT** Freshwater crawfish are cultured throughout the world. There are 3 taxonomic families of crawfish including the Astacidae and Cambaridae in the northern hemisphere and the Parastacidae in the southern hemisphere. There are over 400 species, but widespread commercial aquaculture is limited to no more than 10 species including 3 astacids, *Astacus astacus* Linnaeus, 1758, *Astacus leptodactylus* Eschscholtz, 1823, and *Pacifastacus leniusculus* (Dana 1852); 4 cambarids, *Orconectes immunis* (Hagen 1870), *Procambarus acutus acutus* (Girard 1852), *Procambarus clarkii* (Girard 1852), and *Procambarus zonangulus* Hobbs and Hobbs, 1990; and 3 (4?) parastacids, *Cherax albidus* (Riek 1951) / *Cherax destructor* Clark, 1936, *Cherax quadricarinatus* (Clark 1936), and *Cherax tenuimanus* (Clark 1936). The most important species is *P. clarkii* which accounts for about 90% of the 60-70,000 tons of crawfish cultured annually. While all crawfish species can be cultured intensively, the basic production methods involves establishment of perpetuating populations in monoculture pond systems or self-propagating populations in ricefields. Two forms of semi-intensive crawfish culture have been commercially viable on a small scale. These semi-intensive systems include breeding hatchling crawfish for stocking ponds and producing soft-shell crawfish using pond grown crawfish.

**KEY WORDS:** Crawfish, culture, Astacidae, Cambaridae, Parastacidae

### INTRODUCTION

Freshwater crawfish are cultured throughout the world. There are 3 taxonomic families of crawfish including the Astacidae and Cambaridae in the northern hemisphere and the Parastacidae in the southern hemisphere (Huner 1989). There are over 400 species worldwide. Commercial aquaculture is generally limited to 10 species including 3 astacids, the noble crawfish, *Astacus astacus*, the narrow-clawed crawfish, *Astacus leptodactylus*, and the signal crawfish, *Pacifastacus leniusculus*; 4 cambarids, the calico crawfish, *Orconectes immunis*, *Procambarus acutus acutus*, the red swamp crawfish, *Procambarus clarkii*, and the white river crawfish, *Procambarus zonangulus*; and 3 parastacids, the yabby, *Cherax albidus* / *Cherax destructor*, the redclaw crawfish, *Cherax quadricarinatus*, and the marron, *Cherax tenuimanus*.

There are less than 10 astacid species, more than 300 cambarid species and approximately 100 parastacid species (Hobbs 1988). As interest in freshwater crawfish culture increases, additional species are sure to be cultured. The number of cultivated species will also likely increase as taxonomic problems are resolved with the use of molecular biological techniques. For example, there is confusion about whether or not there are two or more species of yabby and white river crawfish (Huner 1994a, Mills et al. 1994).

This paper will summarize current culture methodology and provide an overview of the global status of freshwater crawfish culture in the mid-1990s. It should be recognized that freshwater crawfish aquaculture is the only large-scale, successful freshwater crustacean aquaculture in temperate regions and is practiced mostly as a component of well-integrated, multi-crop agricultural endeavors.

The companion papers in this series will elaborate on new methodology. Readers are also referred to Huner (1994a) and Hol-dich and Lowery (1988) for additional reviews. Consult Huner (1989) for information about wild freshwater crawfish fisheries.

### PRODUCTION METHODOLOGY

All crawfish species may be cultured intensively from egg to egg in completely closed systems. However, the basic production

methodology involves the establishment of perpetuating populations in monoculture pond systems or self-propagating populations in ricefields. Live, hard-shell crawfish are harvested at sizes less than 15 g for fish bait or 12-150 g for food depending on the species. In general, the most desirable minimum size for food in North America and Europe is 30 g and in Australia is 50 g (Huner 1994a, Ackefors and Lindqvist 1994, Mills et al. 1994).

Two forms of semi-intensive crawfish culture have been commercially viable on a small scale. These include the production of hatchlings and soft-shell crawfish. Hatchling parastacids (*Cherax* spp.) are sold for stocking culture ponds or for the aquarium trade. Hatchling astacids (*A. astacus*, *Austropotamobius pallipes* (Lereboullet 1858), and *P. leniusculus*) are used for restoration of crawfish populations in natural waters. Soft-shell crawfish are used either for fish bait (*Orconectes* spp.) or food, *Procambarus* spp. Broodstock for hatchling production is invariably secured from natural waters or earthen culture ponds. Source crawfish for soft-shell crawfish production units are secured from earthen culture ponds.

Culture in earthen ponds is facilitated by the simple life cycle of all crawfish (Huner and Lindqvist 1991). Following mating, females lay eggs that become attached to abdominal pleopods where they are incubated. Development is direct and the new hatchlings undergo 1 (astacids and parastacids) or 2 molts (cambarids) soon after hatching before assuming the free living form. Technology has been developed for removal of developing eggs for artificial incubation where it is desirable to conserve space in cold water species and/or to produce disease free young.

The most successful commercial crawfish culture has involved those species that retire to burrows to produce hatchlings (*P. clarkii*, *P. acutus acutus*, *P. zonangulus*, and *C. albidus-destructor*) (Huner and Lindqvist 1995). With these species perpetuating populations are established that rarely require any subsequent restocking. Problems with population management invariably result because it has been largely impossible to predict and control densities in ponds. Growth is density dependent and stunting at small, undesirable sizes is frequently encountered. Thus, non-burrowing species including *C. quadricarinatus* and *C. tenuimanus* would

seem more desirable for commercial culture because of the apparent ease of controlling numbers by using hatchery or nursery ponds to produce young. Hatchling production in North America has not been an especially viable commercial option because of high production costs (Medley et al. 1994). Nursery ponds seem to have become an acceptable alternative to costly hatchery systems for Australian producers (Ackefors 1994, Mills et al. 1994).

The North American crawfish culture model involving perpetuating culture of *P. clarkii* and *P. zonangulus* in earthen ponds has been the most successful form of crawfish culture (Huner 1994b, Huner and Barr 1991, Huner and Romaine 1990). Such ponds are 0.3 to 0.5 m in depth and sizes range from 2 to 20 ha. Approximately 50 kg ha<sup>-1</sup> of mature, adult crawfish are stocked in the spring into "new" ponds including ricefields that have not been managed for crawfish during the preceding fall-spring interval. Ponds are then drained with the crawfish surviving and reproducing in burrows during the summer. Vegetation grown in the drained ponds serves as the forage base for a detritus-based ecosystem when ponds are refilled in the fall. A common crop rotation involves cultivation of rice during the summer months with the fields being reflooded for crawfish after the grain is harvested. Vegetative debris from the grain harvest and rice regrowth serve as the plant forage base.

Female crawfish with their young emerge from burrows during the fall after ponds are refilled. Harvesting begins in late fall or early spring and depends on the catch and value of the crawfish. Ponds are drained in the mid to late spring. Restocking is usually not required. Draining of ponds is absolutely essential to ensure establishment of a plant forage base and elimination of predaceous sunfishes (Centrarchidae) and catfishes (Ictaluridae).

As previously stated, control of crawfish numbers is a major problem. The average production is 500 to 700 kg ha<sup>-1</sup> with reported yields as high as 4,000 kg ha<sup>-1</sup> (de la Bretonne and Romaine 1989, Huner and Barr 1991). Restocking of juvenile crawfish from over-populated ponds to under-populated ponds is an effective way to produce predictable crops of large, more valuable crawfish (Huner 1992, McClain et al. 1994). However, this management option has yet to be widely applied because its cost effectiveness depends on the market value which is controlled by the availability of wild crawfish.

Cultured crawfish are harvested with baited traps (see Huner 1994b and other papers in this series). Harvesting is labor intensive and expensive. Active harvesting with net systems or pond draining has proven to be ineffective with the exceptions of very small systems. Typical crawfish ponds have too many obstructions to permit effective use of nets. Furthermore, nets do not discriminate between weak, soft, postmolt, and premolt crawfish and hardy intermolt crawfish. Nets seem most effective in small ponds used to produce smaller, bait-sized crawfish although D'Abramo and Niquette (1991) harvested edible-sized crawfish with a seine in small experimental ponds devoid of emergent vegetation.

There are no well-defined feeding systems for pond cultured crawfish. Most successful culture systems depend on the development of vegetation-based detritus ecosystems (Momot 1995). Popular dogma would have one believe that microbially enriched detritus is the principal source of sustenance for the crawfish. However, Momot (1995) reported that the macroinvertebrate fauna and, perhaps, seeds may be the important food resources. All crawfish will accept pelleted feeds but the use of pelleted feeds has not yet proven to be cost effective in *Procambarus* spp. culture

(Huner 1994a,b). Feeding is practiced to a degree with *Cherax* spp. culture (Ackefors 1994, Mills et al. 1994).

#### SOFT-SHELL CRAWFISH

Soft-shell crawfish are produced by placing intermolt or premolt crawfish, secured from culture ponds, into holding facilities until they molt (Huner 1994b, Culley and Duobinis-Gray 1990). This has been an American phenomenon with smaller soft-shell crawfish [*O. immunis* and *Orconectes rusticus* (Girard 1852)] produced for fish bait in the north-central and north-eastern states and larger ones (*Procambarus* spp.) produced for the gourmet food market in the southeastern states. Malone and Burden (1988) describe elaborate recirculating systems for producing soft-shell *Procambarus* spp. Production of soft-shell crawfish for fish bait in the northern United States has been conducted on a limited scale for over 50 years. Soft-shell crawfish food production in the southern United States began in the late 1980s on a major scale but collapsed in the early 1990s primarily because of high production costs (Louisiana Soft-Shell Crawfish Assoc. 1995). Researchers have developed methods to automate production systems (Malone and Culley 1988) and increase molting rates through bilateral eyestalk ablation (Huner et al. 1993). However, these new technologies came after the industry's collapse and have not been adopted.

#### DISEASES AND PARASITES

Thune (1994) and Alderman and Polglase (1988) provided excellent reviews on diseases and parasites associated with freshwater crawfish. The European astacids and Australian parastacids have long been known to be highly susceptible to the crawfish fungus plague, *Aphanomyces astaci*. This led to the successful introduction of the plague resistant North American astacid, *P. leniusculus* into Europe (Svärdson 1995, Westman and Westman 1992). Because all North American crawfish are assumed to be vectors of the disease, non-resistant species are considered to be at risk in any location where the two come into contact.

A second disease consideration is that of the bacterium *Vibrio*. It has been generally assumed that *P. clarkii* was not susceptible to diseases. However, Thune (1994) presented data to show that *P. clarkii* can suffer high mortality from vibriosis in both earthen ponds and confined holding systems including intensive soft-shell crawfish production units.

#### ECOLOGICAL VALUE OF CRAWFISH CULTURAL SYSTEMS

In the United States, the dominant form of crawfish culture is earthen pond production of *Procambarus* spp. Whether units are managed as mono-crop crawfish systems or multi-crop crawfish/agronomic crop systems, they simulate the natural wet-dry cycle of the region. The ponds are short-hydroperiod wetlands (Huner 1994c) and attract large numbers of wetland-dependent vertebrates, especially birds. Nassar et al. (1991) provided detailed instructions on management of crawfish systems for the attraction of waterfowl such as ducks, coots, and geese. Many predaceous colonial waterbirds are also attracted to crawfish ponds, especially egrets, herons, ibises, gulls, and terns. In fact, there has been a geometric increase in the numbers of egrets, herons, and ibises in southern Louisiana over the past 20 years that has been attributed to the increase in crawfish ponds during the same period (Fleury 1994, Fleury and Sherry 1995). Conflicts have developed between crawfish producers and environmentalists over the impact of avian predation on crawfish crops (Huner 1994c, Fleury and Sherry

1995, Fleury 1994). The elaborate enclosure systems developed to cover Australian crawfish ponds (Ackefors 1994) are too expensive for direct application in the United States. These involve netting, support systems, and peripheral walling.

### STATUS OF CRAWFISH CULTURE

#### *Cambarid* Crawfish

The dominant commercial crawfish species in terms of both culture and natural fisheries is *P. clarkii*. Native to northeastern Mexico and the south-central United States (Hobbs 1988), it has been introduced throughout North America south of Canada and around the world (Hobbs et al. 1989). The United States and the People's Republic of China produce approximately 55,000 and 40,000 metric tons, respectively, with smaller fisheries in Spain and Kenya (Huner 1994b). There is approximately 48,000 ha of culture ponds in the United States with about 95% located in Louisiana. Average production is around 24,000 metric tons (Huner 1994b). Total production in the People's Republic of China is substantially greater than the 2,000 metric tons estimated in the late 1980s (Huner 1989). *P. clarkii* is apparently not cultured intentionally in China but is harvested either from natural wet lands or polyculture systems emphasizing fish production (Xinya 1988, 1995).

Known successful introductions of *P. clarkii* are presented in Table 1. These indicate that the commercial significance of this hardy species is likely to expand in the future.

#### *Parastacid* Crawfishes

Australian crawfish of the genus *Cherax* have attracted much attention from aquaculturists because of their relatively large sizes,

TABLE 1.

Successful introductions of *Procambarus clarkii*.

Caribbean and Central America	Belize
	Costa Rica
	Dominican Republic
South America	Brazil
	Ecuador
	Venezuela
Europe & the Atlantic	The Azores
	France
	Italy
	Netherlands
	Portugal
	Spain
Middle East	Cyprus
	Africa
Asia & the Pacific	Egypt
	Kenya
	Republic of South Africa
	Uganda
	Zambia
	Hawaii
	Hong Kong
	Japan
	People's Republic of China
	Philippines
Taiwan	

References: Huner (1994a), Huner and Barr (1991), Huner (1989), and Hobbs et al. (1989).

over 50 g, when compared to other commercial species (Mills et al. 1994, Morrissy et al. 1995). Initial efforts were devoted to the largest available species, the marron, *C. tenuimanus*. Even though marron have great potential when properly cultivated (Morrissy et al. 1995), they require a mild Mediterranean habitat and high dissolved oxygen levels in the water (Huner and Lindqvist 1995, Morrissy et al. 1990). As a result, there have been no successful commercial aquaculture ventures outside of Australia even though numerous attempts have been made.

Australians have concentrated on the culture of a smaller species called the yabby (or yabbie). The taxonomy is most confused and it may be referenced as *C. destructor*, *C. albidus*, or *C. albidus-destructor* (Ackefors 1994, Mills et al. 1994). The yabby is a burrowing species which tolerates very poor water quality and temperate climates. It has a high fecundity which presents a problem with population control. This situation often leads to stunting with crawfish rarely growing to 50 g (Geddes and Smallridge 1993). There are several hundred hectares of culture ponds devoted to yabby in Australia; however, the "species" has not been accepted worldwide.

Recently, there has been much attention devoted to the culture of the redclaw crawfish, *C. quadricarinatus*, a tropical species with origins in northern Australia. This beautiful crawfish rapidly grows to sizes of 50+ grams and has been introduced into cultural operations on all inhabited continents (Medley et al. 1994). Commercial viability has not been fully tested because of the newness of redclaw development outside Australia. Culture in temperate regions has faced especially acute problems where heated holding systems are required to ensure survival during winters. As a result, production costs have been very high (Medley et al. 1994).

During the 1992–1993 production year, Australian crawfish culture was apportioned as follows: marron, 20 metric tons; yabby, 150 metric tons; and redclaw, 70 metric tons (Smallridge 1994). Marron and yabby were cultured primarily in Western Australia while virtually all of the redclaw were grown in Queensland. Expansion of production efforts in Australia is limited by the availability of suitable land, water resources and markets.

#### *Astacid* Crawfish

Intentional cultivation of astacid crawfish has involved the establishment of perpetuating populations in managed ponds and semi-intensive production of hatchlings or summerlings (juveniles) for re-establishing natural crawfish faunas and/or fisheries (Ackefors and Lindqvist 1994, Pursiainen et al. 1989, Westman and Westman 1992). Järvenpää and Pakke (1995) and Cukerzis (1988) describe methods for artificial incubation and hatching of eggs (developing embryos) from gravid female astacid crawfish. Artificial incubation is done to reduce space requirements where warm water is very expensive to maintain and/or crawfish plague free hatchlings are to be produced.

Gydemo (1995) described earthen pond systems developed to maximize littoral area. Depths range from 1–2 m. However, most European crawfish producers use existing ponds of varying shapes and depths.

The high value of individual, live crawfish in western Europe and Scandinavia has generated much interest in crawfish culture. Wholesale prices for 35–50 g individuals are US\$1 each or US\$20 to 25 kg<sup>-1</sup>. As a result, several hundred crawfish aquaculture licenses have been issued with much interest in England, France, and Sweden. However, the "farms" are generally very small with low production. Production statistics are generally not available; however, it is apparent that fewer than 100 metric tons of crawfish

are actually cultivated in Europe on an annual basis (Ackefors and Lindqvist 1994).

### CONCLUSIONS

Freshwater crayfish are found and cultured on all habitable continents. Very simple technology is required to produce large quantities of several hardy species. However, the extensive production methods now used depend on the establishment of perpetuating populations. As a result, overall production per unit area is

highly variable and generally much lower than has been realized with more sophisticated management practices. Expansion of crayfish aquaculture awaits development of more markets. Those considering the culture of European astacid crayfish and any of the parastacid crayfish must address the risks of having their crayfish stocks destroyed by exposure to the crayfish fungus plague. Entrepreneurs should carefully consider the consequences of the translocation of species outside their natural ranges and possible establishment in natural waters.

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## HARVESTING METHODS AND STRATEGIES USED IN COMMERCIAL PROCAMBARID CRAWFISH AQUACULTURE

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**ABSTRACT** Harvesting of crawfish is the major production cost associated with crawfish (*Procambarus clarkii* and *P. zonangulus*) aquaculture, accounting for 50 to 70% of total direct expenses. Crawfish are harvested for 60 to 150 days with small, baited wire traps, beginning as early as November and generally ending in May-June of the following year. Trap catch is affected by numerous factors including water temperature, water quality, forage and feeding regime, population density and size structure, weather patterns and moon phase, trap design, trap density, number of trapping days, trapping strategy, and bait type and bait quantity. The most effective trap is the pyramid design made from 1.9-cm hexagonal mesh wire-coated with black plastic, and with 3 entrances funnels and an anti-climb collar to minimize crawfish escape. The traps typically are set for 24 hr at a density of 25 to 50 traps per surface hectare (ha) and emptied 3 to 5 days per week. Fish baits, primarily gizzard shad (*Dorosoma cepedianum*) or menhaden (*Brevoortia* spp.), are used as attractants in cold water (<20°C). Formulated baits or a combination of formulated bait and fish bait in the same trap are usually used in warm waters (>20°C). Most producers access traps with flat-bottomed boats propelled with air-cooled engines. Trappers empty and re-bait 150 to 300 traps per hr when motorized boats are used. Improvement in harvesting efficiency can be achieved by developing trap designs that maximize catch while minimizing escape; improving formulated baits that are cost effective in cold and cool water; and modifying trapping strategies that reduce the use of bait and labor without reducing yield. Active harvesting methods such as seines, boat-mounted trawls, or water-flow trapping currently have no commercial application except under specialized conditions.

**KEY WORDS:** Crawfish, aquaculture, harvesting

### INTRODUCTION

Freshwater crawfishes of the genus *Procambarus* are harvested with gear and methods that are not used for most cultured aquatic animals. Most culture species are harvested one to several times per production cycle with seines or by draining the culture system (batch harvesting), or a combination of the two. Freshwater crawfishes are harvested many times for several months with small basket-shaped traps containing bait. Production strategies for culturing freshwater crawfishes in the southeastern United States were reviewed by Huner (1994). An overview of harvesting methods was presented by Romaine (1989), and this review updates more recent developments in harvesting technology.

Harvesting methods and machinery used to trap crawfish has not undergone radical changes since the early 1980s. However, significant improvements in harvesting efficiency have been made in the past decade with the use of better traps, formulated crawfish baits, refinements in trapping strategies, and improved harvesting machinery.

The harvest of crawfish is labor intensive. Typically, 50 to 70% of total direct expenses (TDE) are associated with the harvest. This high cost is because expenses associated with stocking and feeding crawfish are low (Huner 1995). Annual harvest expenses range from \$200 to \$500 per ha, or about \$0.10 to \$0.12/trap/harvest day. Bait (30-55%) and labor to bait and empty traps (20-40%) are the highest expenses. Costs associated with producing and trapping crawfish in Louisiana were reviewed by Baldrige and Huffman (1993). Costs associated with producing crawfish in a 16-ha pond utilizing rice as forage are present in Table 1. Many crawfish producers lease harvest rights to the fisherman, and

trappers receive a percentage of the daily harvest of the daily revenue from the sale of crawfish (de la Bretonne and Fowler 1976). The percentage, normally 40 to 70%, is determined from items supplied by the pond owner and those supplied by the trapper. Trappers who supply their own boats, traps, and bait will normally receive 60 to 70% of the catch or income generated from the sale. The producer might get 50% if he supplies the trapper with bait and traps. Income varies according to seasonal adjustments in crawfish market value and catch.

### FACTORS THAT INFLUENCE CRAWFISH HARVEST

The crawfish catch during harvest is highly variable not only on a seasonal basis but also a day to day basis, varying as much as 2- or 3-fold (Fig. 1). It is not unusual for the catch in ponds to range from 0.1 to 1.5 kg per trap per day during 3 to 5 consecutive trapping days per week over a 6- to 8-month trapping season, that begins as early as November and ends as late as the following June. A seasonal daily average of 0.4 to 0.6 kg per trap is common in well-managed ponds. Long-term seasonal changes in crawfish catch are predictable because it is largely regulated by seasonal changes in water temperature (Araujo and Romaine 1989), but daily changes cannot be forecasted with accuracy. Seasonal and daily variations in harvest are controlled by environmental factors, pond management, and market considerations.

Proper pond design, adequate water exchange capacity, and good water and vegetation management is necessary to maximize crawfish harvest and profit. Water temperature (Hymel 1985, Araujo and Romaine 1989), standing crops of crawfish exceeding 75 mm TL (Momot and Romaine 1983, Araujo and Romaine 1989), and young-of-the-year (YOY) recruitment and molting patterns (Romaine and Lutz 1989) exert the greatest influence on daily catch of *Procambarus*. Crawfish are relatively inactive below

TABLE 1.

Total direct expenses associated with producing procambarid crawfish in a 16-hectare pond planted with rice as forage in southwest Louisiana (Baldrige and Huffman 1993).

Item	\$/Hectare	% of Total
Harvest	375	59
Bait	217	34
Labor	145	23
Miscellaneous	13	2
Water Management	90	14
Forage/Fertilization/Custom	95	15
Repair & Maintenance	62	10
Other	22	4
Total	362	100

10°C, and consequently harvest is lowest in January and February when water temperatures are normally in the range of 10 to 15°C. However, if a large population of holdover crawfish is present from the previous season then mid-winter catches can be high. When water temperature exceeds 20°C in late spring, an increase in crawfish activity and a high number of harvestable-size crawfish results in increased catches, generally in March and April. A significant decrease in daily catch occurs in May and June when water temperature exceeds 30°C, dissolved oxygen (DO) levels frequently decline below 2 mg/L, and when a large portion of the crawfish population matures and burrows. Seasonal change in the crawfish catch in a typical southern Louisiana crawfish pond is depicted in Figure 1.

The extended harvest season for crawfish in managed impoundments occurs from the continuous recruitment of YOY crawfish and holdover crawfish<sup>1</sup> into the population of harvestable-sized animals. When ponds are flooded in mid-September through mid-October, the maximum standing crop of harvestable crawfish, 65 to 75 mm TL or larger, YOY crawfish occurs from mid-March through early May (Romaine and Lutz 1989). If the pond is flooded earlier or later than early October, maximum catch typically shifts earlier or later, corresponding to flooding date (de la Bretonne and Romaine 1989). Higher catch of crawfish in November or December generally reflect a large population of holdover crawfish, although a pond flooded in early September or mid-September, with a corresponding warm fall, may have significant quantities of YOY that reach harvestable size. Because advanced pre-molt or post-molt crawfish cease or reduce feeding activity, they do not contribute significantly to the catch. Crawfish are susceptible to capture when they resume feeding. Crawfish molting patterns, the continuous recruitment of YOY to harvestable sizes, and their subsequent removal from continual trapping, account for much of the cyclic, unpredictable variation in daily crawfish catch.

Crawfish catch is influenced by both short-term and long-term changes in water quality, weather, and moon phase. The physiological stress associated with depressed levels of dissolved oxygen, generally less than 2 mg/L, over a period of 1 to several days decreases feeding activity and subsequent catch (Araujo and Ro-

maire 1989). In commercial ponds, crawfish are frequently exposed to chronically low concentrations of DO for 6 to 12 weeks, particularly in early and late fall (Hymel 1985). Depressed growth and subsequent mortality decreases catch. Rain showers of several minutes to several hours duration increase water circulation and apparently enhance catch by increasing the dispersion of bait attractants (Baum 1987, Araujo and Romaine 1989). Crawfish catch declines with the approach of a full moon and passage of cold fronts (Araujo and Romaine 1989). Overcast weather associated with short duration weather fronts (1 to 3 days) reduces DO concentration and subsequent trap catch. Longer cold fronts (several days or more) decrease water temperature resulting in a reduction in catch. The interaction of dynamic changes in environmental conditions with YOY recruitment and molting patterns is complex and short-term changes in crawfish catch cannot be presently forecasted with accuracy.

### TRAPS

Commercial harvest of *Procambarus* in both native wetlands and aquaculture operations is done almost exclusively with baited traps. Since the 1960s many different traps have been developed and used by commercial fishermen (trappers). Traps differ in configuration, physical dimension, construction material and mesh size, number of entrances, presence or absence of retainer bands or collars, and presence or absence of bait protection containers (Gary 1975, Bean and Huner 1978, Romaine 1983, Pfister and Romaine 1983, Romaine 1988). Most traps are made from 19- or 20-gauge hexagonal mesh poultry-netting coated with black plastic. The plastic coating increases the service life of the trap. The mesh-size opening is 1.9-cm that retains crawfish 65 to 75 mm TL (10 to 12 g) or larger. The inside diameter of the entrance funnels or "flues" is about 3.8 cm. Some fishermen use traps made from 1.6-cm mesh wire to increase catch, but the smaller crawfish retained are not preferred by processors. At present, in Louisiana a mesh size opening of 1.6-cm or smaller is illegal. Plastic-coated square mesh wire has recently become available and is being used in some areas for crawfish traps.

Crawfish traps are designed to either be positioned upright with the top protruding 15 to 20 cm above the water surface (i.e., "stand-up" trap), or lay completely submerged on the bottom (i.e., "pillow" trap) (Pfister and Romaine 1983, Romaine 1983).

### TEMPORAL VARIATION IN CRAWFISH CPUE

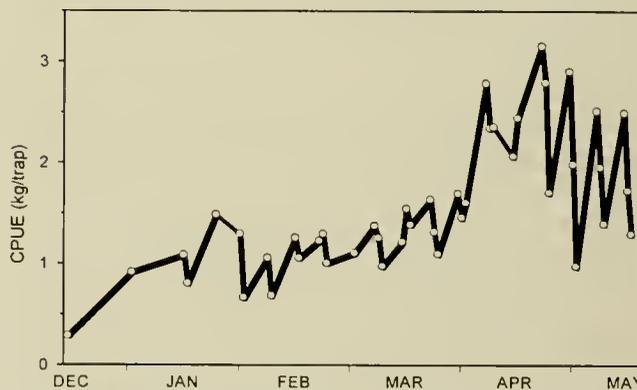


Figure 1. Mean daily variation in crawfish catch per unit trap effort from 2 experimental demonstration crawfish ponds, Aquaculture Research Facility, Louisiana Agricultural Experiment Station, Baton Rouge, LA, 1994.

<sup>1</sup>Holdover crawfish refer to those animals from the preceding production season that were not harvested when the ponds were drained in late spring-early summer.

In shallow-water crawfish ponds, stand-up traps are most frequently used; pillow traps are reserved for water depths exceeding 0.8 m. Stand-up traps are more efficiently lifted, emptied and re-baited than pillow traps, thereby reducing labor costs. Crawfish captured in a stand-up trap with an open top can be emptied, and the trap re-baited, and returned to the water within 15 seconds, whereas 25 to 30 seconds are required to lift, re-bait, and re-set a pillow trap. An additional advantage of stand-up traps is that when DO is low crawfish can climb to the surface and obtain atmospheric oxygen. Submerged pillow traps prevent crawfish from reaching the water surface during DO depletion; this often results in crawfish dying in the trap.

Several design factors influence the catch efficiency of a trap. Traps made from plastic-coated wire and with three-entrance funnels ("flues") have higher catchability than traps made from galvanized wire and with 1 or 2 entrances (Romaine 1983, Pfister and Romaine 1983). Colors of plastic coating other than black do not appear to affect catchability (Romaine 1988). Although traps are coated with plastic to increase their longevity, the slight reduction in the mesh opening from the coating increases catch as much as 25%. Traps with 2 or 3 entrance funnels catch 2 to 3 times more crawfish than traps with 1 entrance (Pfister and Romaine 1983).

Crawfish catch can be increased 15 to 20% by placing a retainer band (7.6-cm wide strip of thin aluminum on the inside circumference at the top of the open area of the trap) or a collar (15-cm dia PVC pipe at the top of the trap) (Fig. 2) at the top of open traps. Retainer bands or collars are also referred to as "anti-climb" devices. Retainers prevent crawfish from escaping from the top of the open trap, but they do not prevent crawfish from escaping through entrances. Depending on the number of funnels, 15 to 20% of the crawfish that enter traps escape within 24 hr by leaving through the entrances, and up to 40% escape after 48 hr (Pfister and Romaine 1983). Smaller crawfish escape more easily than larger animals, so the average size of crawfish caught in traps set for 48 hr is usually larger than those in traps set for 24 hr. Likewise the mean size of crawfish caught after 24-hr trap sets are larger than those caught after 12-hr sets. Traps with bait protection containers (bait wells) catch 40% fewer crawfish than traps with exposed bait (Pfister and Romaine 1983). The inability of crawfish to masticate the bait placed in protection containers apparently decreases the release of attractants within the bait thereby reducing

its effectiveness as an attractant and feeding sounds associated with the mandibles are not made.

Presently, the most effective trap being used by commercial crawfish farmers is the stand-up pyramid trap. The pyramid trap is triangular in shape, has 3 entrance funnels, and a PVC-retainer collar at the top. The collar functions as a handle to lift the trap. The pyramid trap catches 1.4 times more crawfish than the stand-up pillow trap, the second most widely used trap by crawfish farmers (Table 2). Similar results were reported by Huner (1995). The size of each trap design can be varied by using wire of different heights and lengths. Larger traps (e.g., 0.76-m high  $\times$  0.4-m dia) retain more crawfish than smaller traps (e.g., 0.76-m high  $\times$  0.25-m dia) in ponds with a large standing crop of harvestable crawfish because smaller traps are more likely to fill with crawfish. In ponds with low standing crops of harvestable crawfish, smaller traps are as effective as larger traps. Metal rods (1-cm dia) with handles are used to maintain stand-up pillow traps upright. Pyramid traps, because of their wide, flat bottom, normally do not require rods, but wind, and wetland mammals often cause stand-up traps to topple if support rods are not used (Martin and Hamilton 1986).

#### BAIT

Bait is the highest cost associated with producing crawfish (Baldrige and Huffman 1993). Baits generally cost from \$0.31 to \$0.44 per kg, or about \$0.04 to \$0.60/trap/harvest day. Natural fish baits are typically more expensive than formulated bait. The bait cost per hectare varies widely between producers and depends on bait type, quantity used per trap, trap density, and frequency of harvest (days per season and trap sets per day). Fresh bait is used each trapping day and "used" bait is discarded. About 15,000–30,000 tons of bait are used annually in the Louisiana crawfish industry (wild fishery and aquaculture), with nearly half the volume being natural baits and the remainder being formulated.

Natural baits include mostly rough fishes. Clupeid fishes, specifically gizzard shad (*Dorosoma cepedianum*) and Gulf menhaden (*Brevoortia patronus*) are the most widely used natural baits accounting for over 90% of natural baits used. Shad and menhaden are readily available in large supply and at relatively low cost. Herrings (*Alsoa* spp.), common carp (*Cyprinus carpio*), suckers (Catastomidae), and the offal of channel catfish (*Ictalurus punctatus*) and buffalofishes (*Ictiobus* spp.), especially heads, are also used. Clupeids and cyprinids are considered the most effective natural baits (Huner et al. 1990). Formulated baits, that are re-

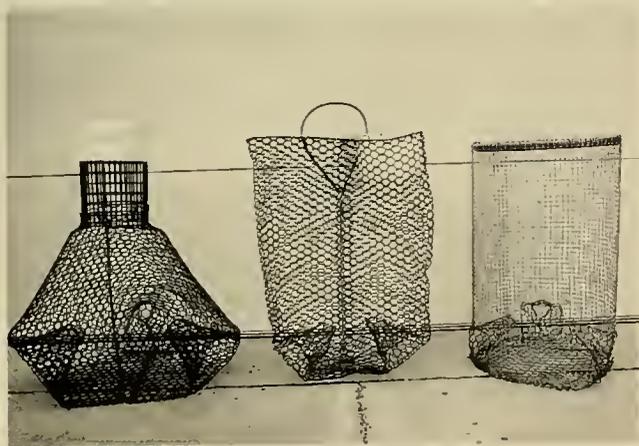


Figure 2. Three types of stand-up crawfish traps used by Louisiana commercial crawfish producers; pyramid (left), stand-up pillow (middle), and barrel (right).

TABLE 2.

Relationship between trap design and trap density on yield of *Procambarus* in ponds at the Aquaculture Research Facility, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, 1990 (Romaine 1990).

Traps/ha	Yield (kg/ha)		
	Pyramid	Stand-up	Mean
30	1,598	1,151	1,375 a
60	2,439	1,659	2,049 b
90	2,660	1,786	2,223 b
Mean	2,232 a	1,532 b	

3 trapping days/week; 44 trapping days/season (Mar–May).

Trap design: 50% pyramid traps and 50% stand-up pillow traps.

ferred to as "artificial" or "manufactured" bait, were developed in the early 1980s. Formulated crawfish baits are made from fish meal, fish solubles, cereal grains, grain byproducts, and commercial flavoring agents. Most formulated baits are cylindrical pellets, 0.4- to 1.25-cm dia and 1.25- to 1.5-cm long, that weigh 50 to 75 g per pellet.

Fish baits are seasonal in supply and price. Large farms may have freezers or coolers for storage, but smaller farms require daily deliveries. Labor is needed to cut the bait that may add as much as \$0.05/kg to the cost of the bait. Shad and menhaden are more effective attractants than formulated baits at water temperatures less than 18–20°C (Fig. 3), and they are used almost exclusively during winter and early spring trapping (Nov–Feb).

Formulated baits are generally less expensive than fish, do not require refrigeration for storage, save labor, and are easier to handle. The most effective formulated baits have a water stability of 12 to 18 hr and a crude protein content of 17 to 20% (Romaine and Osorio 1989, Huner et al. 1990, Huner and Paret 1995). Formulated baits are as effective, or more effective than fish baits at water temperatures exceeding 18 to 20°C, corresponding to March–early April in south Louisiana (Fig. 3). Most brands of formulated bait compare favorably with each other when water temperatures are high and the pond is deficient of forage (Huner et al. 1989, Huner and Paret 1995).

Some trappers add both formulated bait and fish to the same trap. Romaine and Osorio (1989) and Huner et al. (1989) reported that at water temperatures of 20 to 24°C, a fish-formulated bait mixture increased catch by 15 to 30% compared with either the same formulated bait or fish used singly. Huner and Paret (1995) reported only a slight increase in crawfish catch with a fish-formulated bait mixture at temperatures less than 21°C. Beecher (1995) reported that fish-formulated bait mixtures were superior to either formulated bait or fish at water temperatures ranging from 16 to 20°C. Below 16°C, fish (specifically gizzard shad) was most effective, and formulated bait was superior to all other baits at water temperatures exceeding 20°C.

Minimal use of bait is desirable to maximize profit in crawfish aquaculture. Romaine and Osorio (1989) evaluated bait quantities of 150, 225, and 375 g/trap for gizzard shad and several brands of formulated baits in commercial ponds from March through May (water temperatures >19°C). They reported that no increase in

crawfish catch was obtained by using more than 150 g of bait (fish or formulated) per trap. Beecher (1995) evaluated both gizzard shad and a formulated bait at quantities of 100, 150, 225, and 375 g/trap and at water temperatures from 12 to 32°C. Although a slightly higher CPUE was obtained for both shad and the formulated bait with an increase in bait quantity at all temperatures, highest profit was obtained with use of 150 g of bait/trap.

### TRAP DENSITY AND TRAPPING FREQUENCY

Crawfish trappers use from 25 to 100 traps/ha although the industry average is probably no greater than 30 traps/ha. Traps are lifted 2 to 6 days per week, for 3 (March–May) to 7/8 months (November–May/June).

Romaine (1990) reported that crawfish yield increased 49% with an increase in trap density from 30 to 60 traps/ha, but yield increased only 8% with an increase in density from 60 to 90 traps/ha (Table 3). Romaine (1991) further observed that crawfish yield increased 44% with an increase in trapping days from 2 to 3 days every other week (EOW), but catch increased only 17% when trapping effort was increased from 2 to 3 days/week to 4 to 5 days/week (Table 4).

As a further means to reduce harvesting expenses, research has been conducted using a strategy referred to as "rotational trapping." Rotational trapping involves trapping crawfish in part of a pond for 1 to 2 weeks, and then rotating the trapping sequence of the other part for 1 or 2 weeks, and so on, through the end of the harvest season. In theory, this strategy provides crawfish an additional 2 weeks of growth/month in the non-trapped areas of the pond before they are subject to capture by trapping. Two rotational trapping systems, one with supplemental feeding and one without, were compared with 3 days/week trapping (Belhadjali 1994). Trapping effort in rotational trapping was one-half that of 3 days/week trapping. Crawfish yield in 3 days/week trapping (1,896 kg/ha) was 30% higher than rotational trapping with no feed (1,455 kg/ha) and 96% higher than rotation trapping with feed (965 kg/ha) (Table 5).

Based on these findings, a trap density of about 50 to 60 pyramid traps fished 3 or 4 days per week appears to provide highest profit in well-managed ponds with relatively high standing crops of harvestable crawfish. Although a higher yield of crawfish can be obtained with more traps and more trapping days, the additional cost of bait and labor does not justify a higher trapping intensity. Additionally, further research is required on rotational trapping to evaluate its efficacy as a harvesting strategy.

Traps are placed in rows to facilitate harvesting, and the distance between traps depends on trap density. A spacing of 12 to 20 m between individual traps and between rows of traps is most

### BAIT EFFECTIVENESS vs TEMPERATURE

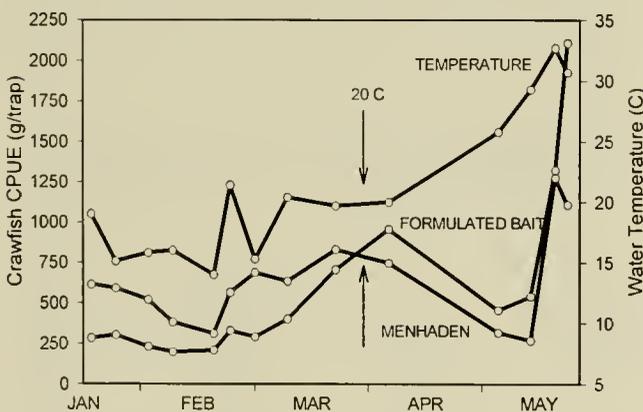


Figure 3. Effectiveness of fish bait and formulated bait in relation to water temperature. Catch data are from 2 experimental demonstration crawfish ponds, Aquaculture Research Facility, Louisiana Agricultural Experiment Station, Baton Rouge, LA, 1993.

TABLE 3.

Effects of trap density on yield of *Procambarus* in ponds at the Aquaculture Research Facility, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, 1990 (Romaine 1990).

Traps/ha	Yield (kg/ha)	CPUE (kg/trap)	Trap-Sets/ha
30	1,375 ± 141 a	1.1 ± 0.1 a	1,320
60	2,049 ± 228 b	0.8 ± 0.1 b	2,640
90	2,223 ± 141 b	0.6 ± 0.1 c	3,960

3 trapping days/week; 44 trapping days/season (Mar–May).

TABLE 4.

Effects of number of trapping days per week on yield of *Procambarus* in ponds at the Aquaculture Research Facility, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, 1991 (Romaine 1991).

Days/Week	Yield (kg/ha)	CPUE (kg/trap)	Mean Harvest Size (g)	Trap-Sets/ha
4 to 5	2,162 ± 286 a	0.9 ± 1.1 a	20 ± 2 a	2,520
2 to 3	1,770 ± 74 b	1.1 ± 0.4 b	28 ± 1 b	1,620
2 to 3/EOW	1,072 ± 94 b	1.1 ± 0.1 c	30 ± 0 b	1,020

Trap density = 60 pyramid traps/ha.

4 to 5 days/week = 42 total trapping days; 2 to 3 days/week = 28 total trapping days; 2 to 3 days/EOW (every other week) = 19 total trapping days.

common. Traps are emptied either from a boat or by walking. Traps are normally emptied within 24 hr of baiting and un-baited traps should be emptied before baiting the traps at the beginning of the following week. Trap-sets of 12 hr catch as much as a third more crawfish than 24-hr sets at water temperatures exceeding 20°C (Romaine and Pfister 1983, Romaine and Osorio 1989). Most producers bait traps in the morning and empty ("run") them the following morning (~24-hr trap set) so that the catch can be brought to market by midday. To increase catch, some trappers empty traps in the morning and re-bait traps later in the afternoon, thereby decreasing the time of the trap-set (~12-hr nocturnal trap set). The number of crawfish declines significantly if the trap is not emptied after 24 hr because many escape.

The size of crawfish caught is correlated with the time traps remain in the water. The shorter the trap set, the higher the number of small crawfish caught. If fresh bait is present in the trap, crawfish of all sizes enter. After the bait has been consumed or the attractants in the bait have been leached, crawfish begin to leave the trap, and escaping is easier for the smaller animals. Even if the trap has retainer bands or collars, many crawfish escape through the entrance funnels. After several days of intense trapping the average size of crawfish harvested decreases (Romaine and Pfister 1983).

Trapping effort affects the size of crawfish harvested. Low trapping effort to reduce harvesting cost may cause a reduction in

growth from depletion of forage and other density-dependent factors (Villagran 1993, Jarboe and Romaine 1995). Stunting can occur, thus resulting in the harvest of small crawfish. By contrast, intensive trapping may reduce harvest size by removing the crawfish before they have had sufficient time to grow to larger sizes. With current management procedures, it is unlikely that mature adults can be overly trapped to reduce YOY recruitment the following production season. However, insufficient harvesting may have an adverse effect on population management. Insufficient harvesting hastens forage depletion, limits food resource availability, and increases intra specific aggression resulting in growth suppression and large standing of small crawfish with low market value (Romaine and Lutz 1989, Villagran 1993). Low trapping effort is often associated with low prices caused by a large wild crop from natural areas. Unfortunately, low trapping effort in ponds with high standing crops of crawfish often leads to excessive YOY recruitment the following production season, thereby compounding the problem.

### HARVESTING MACHINERY

Methods used to empty the traps varies within the industry. Traps may be emptied by trappers who walk shallow-water ponds while pulling a small boat. Harvested crawfish are placed in the boat. One person can empty about 400 traps per day. Producers with ponds less than a few hectares use this method because it is less expensive than purchasing a boat and motor. Other trappers use a small flat-bottom boat that is propelled with a pole or paddle. This method is no more efficient than walking.

A boat propelled with an engine adapted for use in shallow water is the most efficient harvesting equipment. The boats have a flat-bottom, are made from aluminum, and are typically 4.7- to 5.3-m long × 1.2- to 1.5-m wide × 0.46- to 0.61-m high. The propulsion unit is generally an 8 to 12 HP air-cooled engine. One unit used widely in the crawfish industry is the "Go-Devil." The Go-Devil has a long shaft and a weedless propeller, and is designed for use in shallow water (Fig. 4). On some propulsion units the propeller is replaced with a cleated wheel that drags the bottom and pushes the boat forward. A boat powered by a Go-Devil generally requires 2 persons, one to empty and re-bait the traps, and a second to steer. As many as 300 traps per hr can be emptied. Many crawfish trappers use a "crawfish combine" (Fig. 5). The air-cooled engine operates a hydraulic pump and motor that turns a metal wheel(s). Metal cleats are welded to the wheel that is

TABLE 5.

Effects of rotational trapping and supplemental feeding on yield of *Procambarus* in ponds at the Aquaculture Research Facility, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana (Belhadjali 1994).

Treatment	Yield (kg/ha)	CPUE (kg/trap)	Size (g)	Trap-Sets/hz
3/NF	1,830 ± 63 a	0.6 ± 0.1 a	21 ± 8 a	2,580
RT/NF	1,435 ± 81 b	1.0 ± 0.1 b	25 ± 9 b	1,380
RT/FD	970 ± 148 b	0.7 ± 0.1 a	26 ± 9 c	1,380

3/NF = 3 trapping days per week and no formulated feed; RT/NF = rotational trapping-3 trapping days/week and no formulated feed (forage only); RT/FD = rotational trapping-3 trapping days/week and formulated feed used (25% crude protein).

60 pyramid traps/ha; 43 total trapping days.



Figure 4. Crawfish harvest boat, "Go-Devil" design.



Figure 5. Crawfish harvest boat, "crawfish combine" design.

mounted either to the front to pull the boat forward or to the rear to push the boat. A cultivator blade or heavy chain, attached to the side or stern of the boat, is used to prevent the boat from drifting in high winds. The hydraulic system can be operated with foot pedals, leaving the driver's hands free to empty and re-bait traps. A single person can handle about 150–200 traps per hr and up to 300 traps per hr can be emptied and baited with 2 persons.

#### FUTURE DEVELOPMENTS IN CRAWFISH HARVESTING

Present methods for harvesting crawfish are labor intensive and relatively inefficient. The most immediate impact for improving efficiency in crawfish harvesting is to better refine extant harvesting gear and techniques. For example, modification of existing traps or new trap designs that reduce escape of marketable crawfish would enable the trapper to reduce trap density and number of trapping days, thereby lowering labor and bait costs. Development of low-cost formulated baits that are effective in cold and cool water and better methods to more efficiently use bait have high potential to reduce harvesting costs significantly.

Active methods of harvesting crawfish that eliminate the need of traps and baits have been investigated, and although some of these devices have shown promise they are not economical. D'Abramo and Niquette (1991) evaluated seine harvesting of *Procambarus* spp. in ponds. Harvesting of crawfish in the ponds was successfully achieved with seining; however, total yield from

ponds when crawfish were trapped was 1.9 times higher than yield in ponds with seining. Seining was relatively effective because crawfish were fed formulated feed, and no standing macrophytic vegetation was present to interfere with seining. Harvesting crawfish with seines would be difficult in ponds containing vegetation, and the implementation of seine harvesting would likely require a radical departure from current cultivation practices used by commercial crawfish producers.

Boat-mounted push-trawls have been developed and evaluated for harvesting crawfish. Cain and Avault (1983) developed an electro-trawl, and for several years the unit was used to harvest highly valued (\$14 per kg) soft-shell crawfish that do not enter baited traps. Dr. Jay V. Huner and colleagues at the University of Southwestern Louisiana, Lafayette, LA, developed a boat-mounted trawl referred to as a "crawfish skimmer" in the early 1990s. The skimmer, which eliminates the need for traps, was designed for use in the late season when macrophytic vegetative biomass is minimal, crawfish are abundant, and market prices for crawfish are low. The skimmer was at least twice as effective as conventional traps when tested in late spring. For the skimmer to be effective, trapping lanes with no vegetation must be made in ponds before ponds are flooded, and attractants must be placed in the trapping lanes to concentrate the crawfish. The skimmer is most effective in late spring and it is more selective for smaller crawfish than standard commercial traps. Amphibious harvesting units have been developed by entrepreneurs but none have been used on a commercial basis. These units push a front-mounted trawl (see Huner 1994 for a summary).

Use of water-flow trapping techniques that efficiently harvest tropical redclaw crawfish, *Cherax quadricarinatus*, has been reported in Australia. Baum (1987) and Lawson and Romaire (1991) demonstrated that water flow could be used to concentrate crawfish where they can be harvested with large, un-baited hoop traps (1.8-m L × 0.48-m dia). The hoop traps were placed in channels formed by closely spaced (4 m) parallel baffle levees within interior of the crawfish pond. Water currents, generated by flushing the pond with fresh water or by recirculating the water with paddlewheel aerators, stimulated crawfish movement into the channels and subsequently into the hoop traps. Although mean crawfish catch in each un-baited hoop trap was 5 times higher than baited conventional traps, the system was not economical without further improvements and developments in the traps and water circulation system.

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## MANAGEMENT CONSIDERATIONS FOR THE PRODUCTION OF LARGE PROCAMBARID CRAWFISH

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**ABSTRACT** Recent grading practices and substantial price differentials in the Louisiana crawfish industry led to an economic incentive for production of larger crawfish. However, sound management strategies required for the predictable production of large crawfish in commercial ponds have been lacking. Managers have typically concentrated on maximizing total yields with little regard to harvest size. It was once thought that the overwhelming factor causing the excessive production of small crawfish was food shortages. Hays, agricultural by-products, and various feeds have been tried as supplements to the forage-based food system, but results were usually ineffective and not economically feasible. Although it has been demonstrated that total crawfish production can sometimes be increased with prepared feeds, supplemental feeding alone has generally not been effective in producing larger crawfish in pond culture. It has recently been shown that high population density is the single most limiting factor affecting crawfish growth and harvest size, regardless of available nutrition. When crawfish densities were substantially reduced in overpopulated ponds before harvesting commenced, a significant improvement in size-at-harvest was observed. Modifying harvesting strategies also showed potential to increase the average size of crawfish in the catch. By using an intermittent harvest approach as opposed to a continuous approach, crawfish have a greater opportunity to grow to larger sizes prior to their harvest. Relaying or transferring small/stunted crawfish from poor production ponds to underpopulated or new ponds can also significantly increase their size. This has been repeatedly demonstrated in research whereby small, low-value crawfish were transferred late in the season to newly established rice fields and reharvested prior to the grain harvest in an inter-cropping scenario.

**KEY WORDS:** Large crawfish, aquaculture, management

### INTRODUCTION

Recent marketing developments in the Louisiana crawfish (*Procambarus* spp.) industry have caused a major shift in production priorities. Prior to the late 1980s, there was no established pricing system based on a graded and sized product. Pricing was influenced largely by supply and demand with little regard to crawfish size, above the minimum acceptable size of 75 mm total length (Avault et al. 1975) or about 12 g. Therefore, the principal emphasis of management for the crawfish producer was to maximize total production of harvestable crawfish. However, a major export initiative in 1987 (Roberts and Dellenbarger 1989) swayed the crawfish industry to establish grading practices, which subsequently influenced the production and marketing of crawfish.

A decline in the supply of crawfish in Europe opened markets for Louisiana crawfish during the late 1980s (Huner 1989). The export market (mostly to Sweden) was a lucrative market that demanded only select crawfish of the largest size (Harvey 1993). To effectively segregate crawfish for this market, the industry devised various grading processes. The establishment of grading in the industry allowed not only the segregation of crawfish for export but allowed greater developments in the domestic markets, and grading according to size became a standard industry practice (Moody 1989). Louisiana crawfish are now routinely graded into

3 or 4 categories. The largest are used mostly for export, the smallest are used for processing of the abdominal meat, and the medium size(s) are sold mainly as live, ready-to-cook product to restaurants and directly to consumers. Grade category classifications vary depending on geographic region and time of the year; however, the export size category nearly always excludes crawfish smaller than 30 g. Price differentials favor the larger sizes. A recent survey showed prices paid to Louisiana producers for graded crawfish ranged from \$1.74–2.25 kg<sup>-1</sup> for large, \$0.90–1.23 kg<sup>-1</sup> for medium, and \$0.44–0.93 kg<sup>-1</sup> for small crawfish (Landreneau 1995). Also indicated by the survey was that only 27% of the farm production fell into the largest size category, with 32% grading as medium and 41% grading as small (based on 680,000 kg of crawfish). The percentage of small crawfish in the catch can often be much greater for individual producers.

Prior to about 1987, there was little economic incentive for producers in Louisiana to manage for the production of large crawfish. Managers typically focused on maximizing total yields with little regard to harvest size. Likewise, research efforts were directed towards increasing total production. Consequently, the predominance of small crawfish was often associated with high production. Currently, excess production of small, low-value crawfish is a major industry problem and can be economically devastating to individual producers. With the recent establishment of grading practices and market prices proportionally higher for the larger animals, there has been a major shift in production (and research) priority—to increase production of large, high-valued crawfish.

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### FACTORS AFFECTING CRAWFISH GROWTH AND SIZE-AT-HARVEST

Numerous references have been made concerning the excessive production of small, often stunted crawfish (Avault et al. 1975, Huner and Romaine 1978, Romaine and Lutz 1989). Stunted crawfish populations are distinguished by slow growth or a cessation of growth at less than the desired market size of 20 g or larger (Jarboe and Romaine 1995). Although not thoroughly characterized, some of the factors affecting crawfish growth or size-at-harvest are thought to be caused by harvesting strategy, certain water conditions, food availability and food quality, population density, genetic influences, or combinations of these factors (Huner and Romaine 1978). Little information exists of pure genetic influences on growth or size-at-maturity, but most data indicated that environmental factors are more important than genetic effects (Lutz 1987).

The first form of size selection for harvested crawfish is the wire-mesh trap. In Louisiana, 2 mesh sizes are used, 16 and 19 mm, with the latter being the most common (Romaine 1995). The smaller mesh traps retain a smaller crawfish, thus reducing the average harvest size. Harvest intensity can also affect the size of crawfish in the catch. Intense trapping efforts (with high trap density and/or frequent harvests) usually increase overall yields, but can decrease the average size by temporarily decreasing the density of larger crawfish and removing crawfish before they have sufficient time to grow to larger sizes (Romaine and Pfister 1983). Insufficient harvesting can foster overpopulation, which in turn hastens forage depletion and contributes to the stunting of crawfish at sub-desirable sizes (Romaine and Lutz 1989). Trap-set time also can affect the size of crawfish in the catch, because, on average, 15–20% of the crawfish that enter traps escape within 24 hr and up to 40% escape within 48 hr (Pfister and Romaine 1983). Longer sets generally yield larger crawfish because the smaller ones are more effective at exiting the trap.

It is difficult to assess the effects of water conditions on crawfish growth, but they undoubtedly are factors. Chronic low oxygen concentration is thought to suppress growth while rising water temperatures and highly fluctuating water levels are thought to encourage maturation, even at smaller sizes (Huner and Romaine 1978). Yet, acute mortality from oxygen depletion in overpopulated ponds may function to increase crawfish size in subsequent harvests by reducing population density, thus reducing competition for food and space, albeit, total yields may suffer.

It was once thought that the overwhelming factor causing the excessive production of small/stunted crawfish in production ponds was food deficiency. Crawfish production in Louisiana utilizes a forage-based food system (Brunson 1989, Avault and Brunson 1990) in which forage is often depleted before the bulk of the crawfish have been harvested. Harvests of small or stunted crawfish are nearly always associated with premature depletion of the forage. Witzig et al. (1983) found that crawfish harvest weight began declining midway through the trapping season, at which time the mean vegetative biomass had been reduced nearly 80%. Providing hays and agricultural by-products have not been effective in preventing stunting of crawfish in forage depleted ponds (Rivas et al. 1978, Day and Avault 1986). Supplemental feeding of high quality formulated feeds has produced increased yields in some pond studies (Cange et al. 1982, Martinez et al. 1990, McClain et al. 1992, Jarboe and Romaine 1995) but not in others (McClain et al. 1993c, Belhadjali 1994). However, feeding practices frequently proved uneconomical, and positive results were

often inconsistent and difficult to repeat (Romaine 1989). Moreover, supplemental feeds have generally not been effective in inducing a significant growth response in crawfish when used in forage-based ponds, even after forage depletion (Martinez et al. 1990, McClain et al. 1992, Whaley and Eversole 1993, Jarboe and Romaine 1995).

When evaluated under more controlled conditions, high quality formulated feeds (crude protein >20%) were quickly consumed by crawfish and supported rapid growth (Huner et al. 1975, McClain 1995a). Clark et al. (1975) reported that crawfish fed prepared feeds in pools were 39% heavier than crawfish fed vegetative resources (smartweed, *Polygonum* spp. or alligatorweed, *Alternanthera philoxeroides*). McClain (1995b) observed an increase in crawfish weight of 35% and 57% at densities of 2 and 10 crawfish  $m^{-2}$ , respectively, when supplemental feed was offered in addition to detrital-based resources within enclosures. Villagran (1993) reported increased growth with supplemental feeds in pools planted with rice, but only at higher densities (25  $m^{-2}$  or greater).

It has been clearly demonstrated that crawfish exhibit density-dependent growth in which final size is inversely proportional to density (Lutz and Wolters 1986, Villagran 1993, McClain 1995b). Shown in Table 1 is the growth response when *P. clarkii* were cultured in simulated rice-forage production systems at various densities. Growth was profoundly affected by population density in each study and, without exception, harvest weights decreased as density increased. Other studies also demonstrated an inverse relationship between size-at-harvest and crawfish densities (Chien and Avault 1983, Mills and McCloud 1983, Morrissy 1992, Romaine et al. 1978, McClain 1995a).

How growth is affected at high densities has not been fully

Table 1.  
Mean weights at harvest for *P. clarkii* cultured in pools or in enclosures (within ponds) at various densities during 3 different studies.

Initial Density (no $m^{-2}$ )	Weight at Harvest (g)		
	(Lutz & Wolters 1986)	(McClain 1995b)	(Villagran 1993)
1	20.7	—	—
2	14.9	15.3	—
4	13.4	13.8	—
5	—	—	34.0
6	—	11.2	—
8	10.6	—	—
10	—	7.9	32.6
14	—	7.2	—
15	—	—	17.0
16	6.3	—	—
18	—	5.8	—
20	—	—	15.3
25	—	—	12.8
30	—	—	10.5
35	—	—	10.2

Crawfish were not fed formulated feeds but had access to rice detrital food resources. Initial size at stocking was <1 g. (Sources: Lutz and Wolters 1986, McClain 1995b, Villagran 1993).

Lutz and Wolters (1986): 34-day grow out, Jun–Jul.

McClain (1995b): 84-day grow out, Oct–Jan/Feb–May.

Villagran (1993): 175-day grow out, Nov–Jun.

addressed. In forage-based production systems, it is likely that as crawfish density increases beyond some optimum density, indigenous food resources are depleted and nutritional shortages become a limiting factor. However, many studies have found crawfish growth to be density-dependent, regardless of available food supply (Morrissey 1992, Villagran 1993, Brown et al. 1995, McClain 1995a, 1995b). A suspected cause or contributing factor is the social interaction/territorial restriction response that predominates in most species of crawfish (Goyert 1978, Lowery 1988). Notwithstanding, the addition of supplemental feed to a detrital food system has had some mitigating influence on the negative effect of higher crawfish density (Villagran 1993, McClain 1995b). In Table 2, final weights are presented when *P. clarkii* were cultured in separate studies at different densities in rice-based systems with and without formulated supplemental feed. Additionally, a comparison was made between the influence of the feed and the reduction of initial density on final weight at selected culture densities. With each comparison, the increase in final weight from lower initial density was greater than the increase in weight from supplemental feeding. The formulated feed was most beneficial at the higher densities ( $>25$  crawfish  $m^{-2}$ ) (Villagran 1993); although, even high feeding rates have not been able to fully alleviate density effects on crawfish growth (McClain 1995a).

These recent findings suggest that the overwhelming factor affecting size-at-harvest in commercial crawfish (*Procambarus* spp.) ponds, as presently practiced in the southeastern United States, is not primarily food shortages, as was once thought, but principally overcrowding. Although overcrowding can lead to premature forage depletion (Avault et al. 1975, McClain and Romaine, in press) and food shortages, high density apparently inhibits growth even without bringing nutritional limitations into prominence.

## MANAGING CRAWFISH DENSITY

Sound management strategies required for the predictable production of large crawfish in commercial ponds have only recently been studied. Since high population density has been identified as the single most limiting factor affecting crawfish growth and harvest size, managing for proper density should be a top priority in production of large crawfish.

Unlike many other forms of aquaculture that stock known numbers of juveniles, crawfish production in the southeastern United States depends on natural recruitment from stocked or indigenous broodstock. Density of young-of-the-year crawfish in ponds is influenced by many factors (de la Bretonne and Romaine 1989, Huner and Barr 1991), but is a function of the number and size of successfully spawning broodstock and survival of the young. Overpopulation is most likely in permanent ponds after several annual production cycles and is least likely in newly established ponds or in ponds previously out of production for some time (Huner 1994). Therefore, rotating production into different fields, as is commonly used in some rice/crawfish rotational practices (de la Bretonne and Romaine 1989), or allowing a permanent pond to remain temporarily drained after several consecutive cycles can be a management technique for preventing overpopulation when production resumes. However, for those producers with limited land, suspension of production in ponds for an entire season could lead to loss of revenue that might obscure the benefits of population control. Delaying onset of the permanent flood, which usually occurs during September through November in Louisiana to coincide with peak spawning, is another potential method of preventing overpopulation in ponds. Although this action could delay the peak of production (usually accompanied with lower prices), it can sometimes be an effective control measure because it potentially reduces the total number of recruitment classes. Caution

TABLE 2.

Mean weights at harvest for *P. clarkii* cultured in different studies at different densities in rice forage systems with and without supplemental feed. Also presented are increases in harvest weight attributable to the feed or to the reduction of initial density.

	Initial Density (no $m^{-2}$ )	Food Resource		Increase Due to Feed
		(Rice)	(Rice + Feed)	
		McClain 1995b		
	2	15.3 g	20.7 g	35%
	10	7.9 g	12.4 g	57%
Increase due to density reduction		94%	67%	Mean = 46%
		Mean = 80.5%		
		Villagran 1993		
	5	34.0 g	29.5 g	0%
	20	15.3 g	16.0 g	4.6%
Increase due to density reduction		122%	84%	Mean = 2.3%
	20	15.3 g	16.0 g	4.6%
	35	10.2 g	14.9 g	46%
Increase due to density reduction		50%	7.4%	Mean = 25%
		Mean = 29%		

Initial size at stocking was  $<1$  g. (Sources: Villagran 1993, McClain 1995b).

McClain (1995b): 84-day grow out, Oct–Jan/Feb–May.

Villagran (1993): 175-day grow out, Nov–Jun.

must be emphasized with this approach because if cool weather prevails late into spring and/or ponds become deficient of food resources, many late recruits may not have time to attain large harvest size before the ponds are drained in spring (Romaine and Lutz 1989).

When overcrowding is experienced in permanent ponds, a technique to reduce recruitment the following season has been to drain earlier than normal, before too many crawfish become sexually mature and begin mating, and to drain the ponds rapidly, thereby stranding (killing) numerous crawfish before they have sufficient opportunity to burrow. This method is not always effective, particularly in older ponds with many established burrows, and it can potentially cause a loss of income when harvesting ceases prematurely in order to implement the drain.

All of the previously mentioned methods for correcting overpopulation are based on preventative measures, taken after manifestation of the problem during one season, in order to prevent recurrence in a subsequent season. Since the cause of overpopulation is highly variable, largely dependent on environmental factors beyond the manager's control, and is often unpredictable, preventative measures are frequently ineffective, or to the contrary, overcompensatory, and loss of production (and income) is a possibility. Sound management practices required to readily identify overcrowding with corrective actions necessary to yield predictable results within a production season are lacking. However, recent research has focused on this approach and has made some important strides.

Appropriate density levels suitable for acceptable yields but within a range for achieving optimum size-at-harvest have been identified by research to be approximately 10 crawfish  $m^{-2}$  or less under typical forage-based conditions of commercial culture (Lutz and Wolters 1986, Villagran 1993, McClain 1995a, 1995b, McClain and Romaine, in press). Outcomes will depend on many factors, but in general, larger crawfish or greater portions of large crawfish are inversely proportional to density. Accurate assessment of crawfish density in flooded impoundments may not be easily attainable, but relative estimations of population density and size structure may be obtained by use of a seine (Momot and Romaine 1982), dip net (de la Bretonne and Romaine 1989), small-mesh trap (Romaine 1976), or drop sampler (McClain and Romaine, in press), especially when used in combinations. Sampling, beginning 6 to 8 weeks post-flooding, is important in determining population density and potentials for overcrowding. Actions taken to reduce crawfish densities within a current production season, if overcrowding is ascertained, should probably occur after peak recruitment but before ponds warm to near optimum temperatures

in the spring. Reductions before peak recruitment in the fall may not be adequate if large numbers of subsequent recruits appear. Reductions too late in the spring may accompany food shortages and reduced opportunity for maximum growth.

Few research efforts have been directed towards reduction of populations to effect a growth response within a current season. Jarboe and Romaine (1995) were successful at reducing crawfish densities during a production season, but a significant growth response was not achieved. In that study, density was reduced from 10 crawfish  $m^{-2}$  or higher to less than 4  $m^{-2}$ , but mean individual harvest weight was not increased and remained below the commercially desirable size of 20 g or larger. It was surmised that the reduction occurred too late in the season (April) and did not allow sufficient time for further growth of the stunted populations prior to their maturity molt. It was also reported that much of the food resources may have been too severely depleted prior to the density reduction measures; hence, nutritional shortages may have been a contributing factor. McClain and Romaine (in press) were successful in sufficiently reducing crawfish density in overpopulated ponds to increase size-at-harvest in 2 of 3 reduction measures (Table 3). However, only one of the measures (urea application) was effective in achieving increased size-at-harvest without a substantial reduction in total yield. Density reductions occurred in February prior to initiation of the harvest and consisted of: (1) physical removal of crawfish with small-mesh traps; (2) application of urea fertilizer and calcium hydroxide to create ammonia toxicity; and (3) partial draining of ponds (for 5 days) to expose crawfish to hazards such as predation, cannibalism, and cohort aggression. Although midseason reduction measures were very effective in that study, it was stated that corroboration and refinements were needed before management recommendations could be made to crawfish producers. Results of that study, conducted in simulated commercial ponds, primarily revealed how high densities can affect size-at-harvest and that significant increases in crawfish size within a current production season could be achieved by sufficiently reducing crawfish numbers at an appropriate time of the year.

#### MANAGING FOOD RESOURCES

Although population density appears to be the single most important factor determining crawfish growth and size-at-harvest in typical production ponds, limited food resources can also be a significant contributing factor. Moreover, high crawfish density can significantly increase the rate of depletion of the forage in ponds (McClain and Romaine, in press); thus, creating food short-

TABLE 3.

Yields ( $kg\ ha^{-1}$ ) of crawfish (*P. clarkii*) harvested from overcrowded crawfish ponds after implementation of three mid-season density reduction measures (source: McClain and Romaine, in press).

Density Control Measure	Total Annual Yield	Yield by Size Category <sup>1</sup>			
		Grade 1 (Largest)	Grade 2	Grade 3	Grade 4 (Smallest)
No control	1083	24	174	731	154
Physical removal	903	12	78	630	183
Urea application	947	314	327	261	45
Temporary drain	514	116	499	173	26

<sup>1</sup> Crawfish in grade categories 1, 2, 3, and 4 averaged 34.7, 24.2, 16.4, and 11.6 g, respectively.

ages that act to exacerbate density's effect on harvest size. Although, to date, supplemental feeding has had little impact on increasing size-at-harvest in ponds (Martinez et al. 1990, McClain et al. 1992, Jarboe and Romaine 1995), its use at various densities and under varied conditions has not been thoroughly investigated. Aside from any biological benefits of supplemental feeding, feeding must also be economically effective. Use of low cost supplements, such as low-grade whole corn, rice, or soybeans, may provide needed nutritional resources with reasonable economic benefits under certain conditions (Jay Huner, pers. comm.). A well managed forage-based system seems to be an appropriate feed delivery strategy (Avault and Brunson 1990) and remains the basis for the world's largest crawfish aquaculture industry.

There are certain management considerations necessary to ensure maximum utilization of food resources from forage-based production systems. Recent arguments (Momot 1995) suggest that crawfish are primarily carnivores and the true role of a detrital system is to support organisms of animal origin that the crawfish rely on as primary food sources. Avault and Brunson (1990) thoroughly reviewed the use of detrital food systems in crawfish production and emphasized proper management rationales. Probably the single most desirable aspect in any vegetated system is the ability of the forage to continually and consistently furnish adequate amounts of material to the detrital pool for the duration of the 9-month production season. Premature depletion of forage is a major detriment for many crawfish producers (Avault et al. 1975). Research has shown that in ponds planted with rice (the mainstay crawfish forage system), plant maturity is a major determinant for prolonged persistence and regrowth potential (McClain and Dunand 1994). Rice plants that produce a mature seedhead will exhibit a more rapid rate of depletion under a typical fall/winter/spring production season and show less propensity for regrowth in the spring than rice plants that do not reach full physiological maturity. Time of planting and plant variety can significantly influence whether plants reach maturity in temperate climates; many domestic rice varieties will not fully mature if planted after July in Louisiana. Physiological maturity may also influence persistence in other crawfish forage crops, such as the sorghum-sudangrass hybrid. Research is continuing to investigate management approaches to improve forage-based systems and their utilization in crawfish production.

### MANAGING THE HARVEST

Modification of the typical harvesting routine has also shown some potential to increase crawfish size-at-harvest. Intense trapping 5 to 6 days per week can reduce mean harvest size by removing animals from the population before they can grow to a more desirable market size. Romaine (1995) discusses the advantages of using a 3-day per week harvest approach to increase the catch-per-unit-effort and increase overall crawfish size. Other intermittent or rotational harvest schedules used to increase efficiency of the harvest have also been shown to increase the proportion of large crawfish in the catch (McClain et al. 1993c, Belhadjali 1994). When an entire pond or portions of a pond are harvested on an intermittent basis, crawfish have an opportunity to undergo additional molt(s), thus increasing crawfish size and value between trapping episodes. Various periods of trapping/non-trapping, as well as use of supplemental feeds during the non-trapping period, have been investigated. McClain et al. (1993c) used a trapping period of 1 week followed by 3 weeks of non-

trapping. Belhadjali (1994) used a 2-week trapping period followed by 2 weeks of non-trapping. Additions of formulated feeds during the non-trapping period in those 2 studies did little to increase yield or size-at-harvest. Generally, intermittent trapping has increased crawfish harvest size but at the expense of total yield, probably from decreased trapping frequency (i.e., less trap-sets per season). Increased trapping intensity during periods of harvest in an intermittent strategy by increasing trap density may result in larger crawfish without a significant reduction in total yield (Romaine 1995).

The efficacy of an intermittent harvest schedule will depend on many factors, such as population density and size structure, food availability, time of year, trap density, cyclic schedule, and marketing conditions. As the technique becomes better refined in relationship to combinations of those factors, an intermittent harvest is likely to be an effective management tool routinely used for increasing the proportion of large crawfish in the catch. Research institutions, as well as individual producers, are continuing to explore different trapping strategies for their effectiveness.

### RELAYING TO INCREASE CRAWFISH SIZE

The vast majority of small crawfish occur near the end of the production season (late April-early June in Louisiana), which normally coincides with the period of lowest prices. Vegetative food resources are frequently depleted by that time, often as a result of high population densities. Supplemental feeding and/or density reduction, when implemented late in the season, have failed to stimulate a growth response (Jarboe and Romaine 1995). Only by transferring, or "relaying", crawfish from their original surroundings (i.e., a poor environment) to an improved environment, has it been possible to achieve further growth from severely stunted populations. McClain et al. (1993a) reported that small/stunted crawfish (mean weight = 15 g) relayed into a newly established rice field and reharvested after about 30 days doubled in weight with up to 52% (of the total yield) grading into the largest, high-value size category upon harvest.

The emphasis on relaying research was to establish a practice whereby producers could utilize an existing situation, common to many farms, to increase the market value of crawfish, extend the harvest season, and increase net returns from an integrated agriculture operation. Because of common resource requirements, crawfish culture is frequently used by rice producers in crop rotational practices (de la Bretonne and Romaine 1989). Because crawfish and rice seasons overlap to some degree, it is common to have newly established rice fields at a time when crawfish stunting normally occurs in forage-depleted crawfish ponds. This provided the opportunity of utilizing the vegetative growth phase of rice production as a valuable resource for obtaining additional growth and increasing the market value of crawfish. McClain et al. (1993a) reported that good rice yields were obtained following the crawfish harvest, and potential net farm income was increased up to three-fold by intercropping crawfish in a rice crop. Subsequent investigations (McClain et al. 1993b, McClain et al. 1995) further examined this intercropping approach at stocking densities of stunted crawfish ranging from 280 to 1,120 kg ha<sup>-1</sup> and consistently found crawfish size to increase markedly after relaying, with average size-at-harvest dependent on initial size at stocking and stocking density.

Integrating crawfish and rice in an intercropping approach (Fig. 1) demonstrated to be effective in substantially increasing crawfish



Figure 1. Harvesting crayfish that were "relayed" into a rice crop in order to achieve further growth (thus increasing marketing value) during the vegetative growth phase of rice production—an intercropping approach.

size and value, which potentially provides for favorable economic impacts to farming systems that are already integrated. The transferring, or relaying, process may also be an appropriate management strategy for less integrated systems and at other times of the year. Huner (1992) reported that supplemental stockings of small ponds in February and March yielded large crayfish. Producers have also reported a positive response when small crayfish from overpopulated ponds or other areas were relayed (at various times of the year) into crayfish ponds that had crop failures. Another possibility, that has not been thoroughly investigated, is the potential to relay culled or small, low-value crayfish from grading apparatuses to pond production systems for further growth. With

careful handling and if done in a timely manner, this approach should be biologically effective; however, it should be closely scrutinized for economical efficacy.

The economical efficacy of relaying or any other management practice intended to increase the production of large crayfish in a commercial operation depends on a differential price to the producer that favors larger crayfish. The magnitude of that differential will largely dictate the feasibility of management practices beyond those that are necessary for optimum yields of medium-size animals. Market demands and price differences should always be taken into account when implementing or recommending costly management practices.

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## A REVIEW OF NUTRITIONAL RESEARCH WITH CRAYFISH<sup>1</sup>

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**ABSTRACT** Culture of crayfish is one of the few industries that developed without significant nutritional information. However, there is an increasing interest in developing precise nutritional information for the commercially important species of crayfish. Nutrient absorption values indicate that plant protein feedstuffs might be good choices for practical diet formulation; processing removes potentially available nutrients from plant feedstuffs. Most digestibility or absorption values indicate relatively efficient use of ingested nutrients. Qualitative nutritional requirements are largely known while only a few requirements have been quantified. Crayfish, like other crustaceans, have some atypical nutritional requirements such as asparagine, cholesterol or phytosterols, phosphatidylcholine, and ascorbic acid. Experimental diets are still under evaluation and standardization has not been achieved. Initial practical dietary formulations have been modifications of diets fed to fish. Feeding those diets results in increases in production; however, the additional cost may not be justified. Diets for crayfish have been simplified in recent years to individual feedstuffs fed as supplemental food in pond culture systems. Formal evaluations of naturally occurring food items verified that crayfish can use bacteria as a source of food, but that zooplankton is an important food source for first-feeding crayfish. Nutritional studies with crayfish are relatively new lines of research, which should benefit the various crayfish industries as more data are developed.

**KEY WORDS:** Crayfish, nutrition, review

### INTRODUCTION

The crayfish aquacultural industry verges on uniqueness. No other animal production industry developed in the modern ages without information regarding optimum nutrition for the target species. Yet, a significant crayfish industry developed in the United States in the latter half of this century and the uncertainties about optimal nutrition for crayfish remain. This is a tribute to the generalized feeding habits of crayfish. However, numerous beliefs developed during this time on foods of crayfish and many of those beliefs became accepted. Only recently, with the advent of controlled nutritional studies, have those beliefs been modified based on published data and changed our broadly generalized perception of crayfish.

Most aquaculturists and biologists would argue that crayfish eat anything and everything they encounter in an aquatic ecosystem. Indeed, the list of foods consumed by crayfish in natural settings has been expanding for the past century (Brown 1995). This review focuses on recent nutritional data developed for crayfish and the beginnings of differentiating normal pond biota into nutritionally important foods.

### NUTRIENT DIGESTIBILITY

One of the fundamental concepts in animal nutrition is the ability to digest and absorb nutrients once identified and consumed. Crayfish have ample capability of locating foods and significant reduction in food particle size occurs after acquisition (Brown 1995). Enzymatic activities and reductions in pH further facilitate catabolism of food into components that can be absorbed. Based on a series of studies with crayfish, relatively specific guidelines were developed for conducting nutrient digestion or absorption studies. The methods used in those studies clearly identified differential absorption of nutrients and preferences for foods.

### Methods

There are 2 general types of nutrient absorption studies; the indicator and total fecal collection methods (Schneider and Flatt 1975). The indicator method is most commonly used with fish, with chromium and other compounds used most (Wilson et al. 1981, Brown et al. 1985, Hajen et al. 1993, NRC 1993, Riche et al. 1995). The total fecal collection method requires quantitative monitoring of feed intake and collection of feces, whereas the use of an indicator does not require precise measurement of feed consumption.

A preliminary study with lobsters (*Homarus americanus*) reported the indicator method was inappropriate because it violated several of the assumptions that must be made when working with an indicator (Leavitt 1983). Probably the earliest nutrient absorption study with crayfish used the total fecal collection method (Wiernicki 1984). Brown et al. (1986) compared the total fecal collection method with the indicator method for use with *Procambarus clarkii*. Chromic oxide was used as the indicator. In that study, chromium violated two of the assumptions regarding appropriate use of indicators. Chromium was not consumed in the same proportion as the remainder of the diet and the fraction that was consumed moved through the gastrointestinal tract at a more rapid rate than the remainder of the diet. Crayfish, through normal food acquisition activities, were able to break the feed pellet into small pieces and selectively consumed the preferred ingredients, leaving the indicator. Movement of the green chromic oxide at a more rapid rate was visually observed and might have been a function of the density of chromium relative to the remainder of the diet coupled with significant mixing that occurs in the stomach. Both factors would preclude calculation of a realistic digestion value. Other indicators might be useful, but have not been evaluated. Based on this 1 comparison, the total fecal collection method should be the method of choice until further evaluations identify other appropriate methods.

A fecal collection chamber was developed that was two separate, but attached, 1.0-L containers (Fig. 1). The containers were stacked on top of each other. The top container held the crayfish

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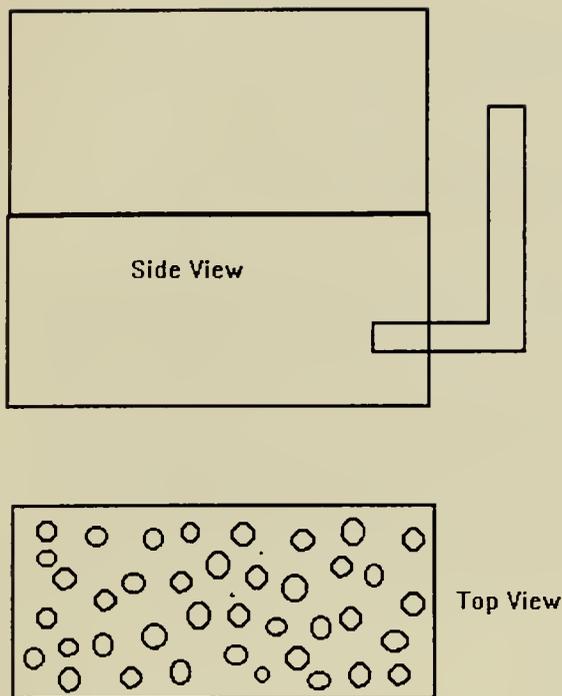


Figure 1. Schematic side and top view of the fecal collection chamber developed for crayfish.

and the bottom of that container was perforated with holes (0.64 cm diameter). Individual crayfish were placed in the top container. The bottom container was equipped with an external standpipe that regulated the water level in the top chamber. Crayfish remained in the top chamber throughout each day, being removed only during the period that meals were offered. Thus, feces fell through the floor of the top chamber and settled in the bottom chamber. Movement of fecal samples from the settling chamber was inhibited by 0.45  $\mu\text{m}$  screen placed on the outside of the standpipe. This approach minimizes the potential of coprophagy. Further, this results in measurements of consumption and nutrient absorption for individual crayfish.

A digestion trial can be divided into 2 separate, but related components. The preliminary period is the time for the experimental animal to adjust to the conditions of the experiment and the diet offered, normally 4–6 days for nonruminant animals. The second component is the fecal collection period. Duration of this period is variable but typical recommendations are 4–10 days (Schneider and Flatt 1975). In their first studies, Brown et al. (1986, 1989) used a 6-day preliminary period and 4-day fecal collection period. A later study concluded that a 6-day preliminary period was appropriate for adjustment to a new diet (Brown et al. 1990). When evaluating several feedstuffs or diets, digestion trials are often conducted sequentially with the same experimental animals. Consistent consumption of the test feedstuff or diet is an important factor. For example, gorging behaviour has been described in crayfish (Rundquist and Goldman 1983). When crayfish are offered a new feed, increases or decreases in consumption may occur, which will not be reflective of digestion values determined during consistent consumption of the same diet. Appropriate duration of fecal collection has not been formally evaluated. Note that fecal collection periods were for a full 4 days. For example, feedings continued for 4 days, with the last fecal sample collected on the morning of the fifth day.

During both the preliminary and fecal collection periods, meals were offered to individual crayfish twice per day. At each meal, crayfish were removed from the fecal collection chamber and placed in a single 1.0-L chamber. The feeding area was an identical container to those used in constructing the fecal collection chamber, but without a perforated floor. Crayfish were placed in the feeding chamber and the food was added. All crayfish were allowed 1 hour for consumption of the meal. After 1 hour, crayfish were carefully removed, washed with a spray bottle, and placed back into the fecal collection chamber. During the fecal collection period, all meals were preweighed and of a known moisture content. All fecal pellets excreted in the feeding chamber were removed and added to the fecal collections for that period.

During meals, fecal samples were quantitatively removed from the collection chamber, filtered initially on a 0.45  $\mu\text{m}$  screen, then transferred to preweighed aluminum drying pans. Samples were then dried at 100°C and weighed. Later studies used preweighed filter paper and vacuum filtration for collecting both uneaten food and feces. Once dried, both feed and feces were chemically analyzed by standard procedures. Usually, microanalytical techniques were employed. Even after 4 days collection, composite fecal sample weights for individual crayfish were less than 0.25 g. Dry matter digestion values were then simply the sum of ingested food minus the sum of feces. For specific nutrients, multiplying food and fecal weights by their respective nutritional concentrations provides the appropriate values for subtraction.

One of the obvious unanswered questions is the effect of leaching on nutrient absorption values. Crayfish fecal pellets are distinct in the fecal collection chamber, even 12 hours after the last collection, and seem to be enveloped in a mucosal-like membrane, which should inhibit leaching. Leaching from feed samples was accounted for in each of the previous studies by placing a known weight of feed in water for 1 hour and measuring loss of dry matter and specific nutrients. Those values were used to correct both consumption and nutrient absorption values.

Reigh et al. (1990) used a modified approach. In their studies, 4 crayfish were held in an individual tank segregated by netting. Crayfish were fed in their respective compartments and uneaten food was siphoned from the tank 30 minutes after feeding. Feces were siphoned four times per day. No preliminary period was conducted and the fecal collection period was 3 weeks. More frequent collection of fecal material should be viewed as a positive modification. However, if crayfish are coprophagous, leaving the test animal in close proximity to their feces would lead to erroneous values.

Ellis et al. (1987) placed finger cots over the posterior end of *Pacifastacus leniusculus* for collection of feces and used chromic oxide as the indicator. Crayfish were fed a series of commercial diets. They employed a 3-day preliminary period and 2-day fecal collection period.

#### Values

Using the methods outlined above, several nutrient absorption values were developed for crayfish. Those values and others are presented in Table 1. Several pertinent generalizations can be drawn from these data.

First, crayfish appear to have distinct preferences for selected feedstuffs, particularly those plant feedstuffs containing relatively high levels of nitrogen-free extract (NFE, or soluble carbohydrate). Additionally, apparent dry matter digestibility was gener-

TABLE 1.

Mean consumption (% of body weight per day), and nutrient absorption values for crayfish.

Feedstuff	Consumption	Nutrient Absorption Value (%)			Reference
		Dry Matter	Crude Protein	Energy	
<i>Procambarus clarkii</i>					
Casein/Gelatin	nd <sup>1</sup>	nr <sup>2</sup>	>90	nd	Nose 1964
Albumin/Gelatin	nd	nr	>90	nd	"
Elodea ( <i>Egera densa</i> )	nr	20-45	nd	nd	Wiernicki 1984
Rice Bran	2.7	88.6	91.8	nd	Brown et al. 1986
Wheat Bran	1.5	78.7	95.7	nd	"
Soybean Meal (Sol. Ext.)	1.3	80.5	98.7	nd	"
Chitin	1.2	71.6	68.3	nd	"
Casein	0.9	73.4	95.4	nd	"
Fish Meal (Menhaden)	0.6	61.1	85.4	nd	"
Wheat Gluten	0.6	63.5	95.0	nd	"
Shrimp-head Meal	0.4	39.1	81.2	nd	"
α-Soy Protein	0.4	25.0	95.9	nd	"
Alfalfa Meal	nr	34.7	75.6	35.6	Reigh et al. 1990
Corn Grain	nr	100.8	97.3	100.0	"
Cottonseed Meal	nr	64.8	83.7	67.9	"
Crab Meal	nr	61.9	81.1	68.1	"
Meat and Bone Meal	nr	61.3	76.5	78.1	"
Fish Meal (Menhaden)	nr	71.6	80.7	82.5	"
Milo	nr	87.5	86.0	82.7	"
Rice Bran	nr	71.0	93.5	73.7	"
Rice Grain	nr	70.8	87.7	70.8	"
Soybean Meal	nr	78.6	94.8	83.1	"
Wheat Flour	nr	103.5	96.4	99.5	"
Wheat Gluten	nr	101.7	103.2	100.9	"
Wheat Midds	nr	74.8	92.5	75.3	"
Wheat Shorts	nr	81.6	92.2	79.5	"
Yeast	nr	61.6	69.8	55.3	"
<i>Pacifastacus leniusculus</i>					
Shrimp Feed (40% CP <sup>3</sup> )	nr	nr	97.4	91.5	Ellis et al. 1987
Shrimp Feed (30% CP)	nr	nr	95.8	80.4	"
Trout Feed	nr	nr	99.0	nd	"

<sup>1</sup> Not determined.<sup>2</sup> Not reported.<sup>3</sup> Crude protein.

ally higher from feedstuffs that had not been further processed. For example, processing of soybean meal and wheat midds or bran to soy protein concentrates and wheat gluten, respectively, removes a significant portion of the NFE and a significant portion of the potentially available dry matter. Thus, NFE appears to be important to crayfish both in terms of consumption of feedstuffs and as a source of available nutrients. Most of the crude protein and energy absorption values developed have been relatively high, which is similar to data for fish. The most obvious exception has been chitin.

Chitin is a complex structure, containing a significant amount of nitrogen as N-acetylglucosamine. Crude protein is simply a measure of nitrogen (N), which is then multiplied by a standard value of 6.25 to estimate the protein concentration. Though chitinase has been identified in crayfish (Musgrove 1988, Brown 1995), the ability of crayfish to degrade ground chitin from a pure source, shrimp-head meal or crab meal appears limited. It is possible that other factors contributed to the relatively low dry matter digestibility values for feedstuffs containing relatively high levels of chitin. Similarly, the N in chitin is not an amino acid; thus, limited chitinase activity may result in partial catabolism of chitin

leaving a relatively high level of N as glucosamine units or unbound amines. This scenario would result in higher fecal N concentrations and lower crude protein absorption values.

The most surprising results from the studies presented in Table 1 are the consumption and nutrient availability values for menhaden fish meal. Menhaden meal was poorly consumed (0.6% body weight) and the dry matter digestibility values were 61-71%; apparent crude protein absorption values were 80-85%. Both sets of values are relatively low when compared to similar values for plant feedstuffs. Whole fish was the preferred bait for harvesting crayfish from culture ponds for many years and remains a popular bait at certain times of the year. Thus, it seems clear that crayfish are attracted to whole fish. However, they do not seem to prefer a processed fish meal made from menhaden. Crayfish producers feel certain that preferences for fish used as bait change with seasons, which is probably a function of water temperature and possibly rate of degradation in the trap. Thus, 2 potential factors may have led to the relatively low consumption and nutrient absorption values for menhaden fish meal. Processing of whole fish to fish meal involves drying, grinding and pressing. This removes both water- and lipid-soluble components that may be flavor attractants for

crayfish. The other potential factor is that crayfish simply do not prefer menhaden meal. Fish meals from other sources have not been evaluated, yet.

If we compare the nutrient absorption values using the 2 methods described above, we see some similarities. For example, apparent dry matter and crude protein digestibility values were similar for soybean meal and most of the apparent crude protein values were similar. However, several of the values were distinctly different. Methods used were slightly different as described above and that may have contributed to the dissimilarities. Further, experimental diets were not similar.

There are 2 basic dietary formulation approaches for determining nutrient absorption values. One approach uses the test feedstuff as the sole ingredient in the diet or the sole source of the specific nutrient of interest. That approach was used by Brown et al. (1986). The other basic approach is to incorporate the test feedstuff into a reference diet at a constant level of substitution, usually 30%. That approach was used by Reigh et al. (1990). Both experimental approaches have positive and negative aspects. Brown et al. (1989) compared the 2 methods and reported significant differences depending on the approach (Table 2). Thus, interpretation of consumption and nutrient absorption data should be made with caution. For example, culturists using supplemental feeding regimes that might use single ingredients as inputs into pond culture systems should use those values developed with a single ingredient, while those feeding crayfish in more intensive indoor systems should probably use data developed from multi-ingredient diets.

The only apparent amino acid availability values were developed by Ellis et al. (1987). Apparent alanine and lysine availability values were 98.5 and 95.0%, respectively, which were similar to their apparent crude protein digestibility values. Crayfish were fed commercial shrimp feeds supplemented with 3% of each amino acid.

## NUTRITIONAL REQUIREMENTS

Requirement of animals for specific nutrients is another fundamental aspect of nutrition. Qualitative requirements simply imply that the animal requires the specific nutrient in their diet, while quantitative requirements recommend a specified amount of the nutrient. Currently we have few quantitative nutritional require-

ments for crayfish, although methods used have become relatively standardized.

## Methods

Freshwater crayfish are cannibalistic. Rearing crayfish at densities above 5 individuals  $m^{-2}$  typically results in significant mortality (Brown et al. 1995). Ingestion of cohorts is a serious confounding effect in any nutritional study. Thus, the most commonly used experimental approach for determining nutritional requirements is to house crayfish in individual containers. Further, crayfish should have an independent water supply operated as a flow-through system. Chemical communication occurs between crayfish (Hazlett 1985a,b, 1989, 1990). Response of subordinate crayfish to the presence of dominant crayfish has been reported as inhibiting in nature. If dominant crayfish are in a recirculating system, even if they are in their own container, circulating chemicals might inhibit normal consumption of food offered to subordinate animals.

Feed is offered to each animal at some regular interval, often twice per day. Juvenile crayfish are most commonly used because they are young and growing at a rapid rate. Nutritional requirements developed for young, rapidly growing crayfish should be adequate for larger animals. Working with individual animals that exhibit relatively high variability in growth within a cohort demands some changes in sample size and feeding strategy.

D'Abramo and Robinson (1989) recommended greater than 30 individuals per dietary treatment. Most studies conducted to date used between 20 and 30 per treatment and recorded significant treatment effects. Feeding large numbers of juvenile crayfish individual meals at a restricted feed rate (e.g., as a percentage of body weight per day) is impractical. Thus, most studies adopted an "in excess" feeding strategy. That is, some consistent amount of feed was offered to each crayfish. That amount should be held as constant as possible for each replicate within each treatment and between treatments. Often, experimental diets are sieved to a relatively uniform size and equal numbers of feed pellets are offered. The amount should be in excess of what the crayfish could eat in a 15–30 minute period. The obvious weak point in this experimental design is that differences in consumption between diets cannot be determined. The methods described above should be accepted standards for conducting precise nutritional research with crayfish. However, there are other experimental designs that have some practical utility.

Crayfish reared in ponds and those reared in recirculating systems for soft-shell production are usually not placed in individual containers, but are reared in communal groups. Thus, some experimental designs using that approach would be pertinent for practical diet development and can yield useful information (Morrissy 1984, D'Abramo et al. 1985, Celada et al. 1989).

Diets used in quantitative nutritional studies with crayfish have not been standardized and typically result in relatively low weight gain compared to crayfish reared in commercial culture conditions. Most of the early diets used were simply modifications of experimental fish diets containing casein, gelatin, dextrin, and vitamin premix and mineral premixes. Those diets were accepted by juvenile crayfish and dietary treatment effects developed over the course of 8–12 weeks. Wetzel (1993) provided an indication that formulation strategy could be improved.

In a controlled study, juvenile *Orconectes virilis* were fed several diets. Those diets included the Standard Crustacean Reference

TABLE 2.

Mean consumption (% of body weight per day), apparent dry matter digestibility (ADMD) and apparent digestible energy (ADE) for feedstuffs fed to red swamp crayfish either substituted into a reference diet (mixed) or as a single ingredient (single).

Feedstuff	Consumption		ADMD		ADE	
	Mixed	Single	Mixed	Single	Mixed	Single
Soybean Meal	1.0	0.4	98	68	91	89
Peanut Meal	0.6	1.2	114	89	108	89
Wheat Shorts	0.8	0.9	94	82	84	87
Cooked Corn	0.7	0.3	108	85	94	90
Fish Meal (Menhaden)	0.3	0.7	–5	91	28	70
Shrimp-head Meal	0.5	0.2	71	41	78	85
Reference Diet	0.8	–	85	–	88	–

Diet (SCRD) (Castell et al. 1989). The basal diet contained casein, gelatin and an amino acid premix containing both essential and nonessential amino acids. Crayfish fed a diet containing glucose-amine as a source of carbohydrate instead of dextrin plus supplemental asparagine gained almost double the weight of crayfish fed the SCRD. This is similar to data of Morrissy (1989) for *C. tenuimanus*. Thus, it could be argued that experimental diets for nutritional research with crayfish have not been fully explored.

### Requirements

Using the methods described above, several estimates of nutritional needs of crayfish have been reported. Even if these values were developed by feeding suboptimal experimental diets, they are useful in diet formulation. The current estimates, though, may be modified as we learn more about nutrition of crayfish.

The current qualitative nutritional needs of crayfish are presented in Table 3. Qualitative essential amino acid needs of crayfish include the typical ten amino acids required by most animals plus asparagine. Van Marrewijk and Zandee (1975) initially reported the need for asparagine in *Astacus astacus* by injecting labeled precursors. Recently, Wetzel (1993) demonstrated that asparagine was required for growth of *O. virilis*. Juvenile crayfish fed a supplemental source of asparagine in purified diets gained approximately twice the weight of those fed an identical diet without asparagine supplementation. Crustaceans, including crayfish, require several atypical lipid-soluble nutrients.

Cholesterol or phytosterols (D'Abramo et al. 1985), and lecithin or phosphatidylcholine (Lochmann et al. 1992) are both re-

quired by crayfish. The need for cholesterol has been demonstrated both by administration of labeled precursors and growth studies, while the need for lecithin has been demonstrated by growth study. Lochmann et al. (1992) could not demonstrate a dietary cholesterol requirement for *P. clarkii* fed practical diets. Differences in dietary formulations or species may explain this observation. Linoleic acid (18:2n-6) could not be synthesized from labeled precursors (Zandee 1966) and should be considered an essential fatty acid. D'Abramo and Robinson (1989) speculated that linolenic acid or linoleic acid may be essential fatty acids. Other lipid-soluble components are most likely required by crayfish, but have not been evaluated. Similarly, there have been few evaluations of the need for vitamin or mineral supplementation in diets fed to crayfish.

Juvenile *O. virilis* were fed purified diets with or without ascorbic acid (vitamin C). Those fed the diet without vitamin C exhibited reduced weight gain and significantly higher mortality (unpubl. data). Mortality occurred during ecdysis. In a separate study, juvenile *O. virilis* were also fed a series of vitamin A sources (retinyl acetate, retinoic acid, retinal, retinol and  $\beta$ -carotene) supplemented at similar levels of vitamin A activity in purified diets. Those fed  $\beta$ -carotene exhibited normal pigmentation after 6 weeks of feeding, while those fed all other sources exhibited either a blue color or complete lack of pigmentation in the exoskeleton (unpubl. data). Atypical pigmentation is relatively common in crustaceans fed many of the practical or purified diets, and emphasizes the need for additional research into appropriate diets. Lack of vitamin A did not influence weight gain in crayfish. This is a similar finding to those of Celada et al. (1989) for *P. leniusculus*. Lochmann et al. (1992) reported improved weight gain of *P. clarkii* fed astaxanthin compared to weight gain of crayfish fed  $\beta$ -carotene. Vitamin A-ester concentrate was also in each of those diets. Similarly, results from other studies indicated normal pigmentation of both exoskeleton and hepatopancreas in crayfish fed various macrophytes (Huner and Meyers 1979, Huner 1984). There have not been other specific vitamin studies with crayfish.

Phosphorus (P) is often the first limiting mineral in diets fed to freshwater fish, but has not been established as essential in diets fed to crayfish (Mayeaux 1988). When fed practical diets, *P. clarkii* seemed to require supplemental P in one study, but not in another (Lochmann et al. 1992). There is a clear need for environmental calcium for crayfish, but no studies with dietary calcium or the interaction of the two. No other mineral nutrition studies have been conducted with crayfish.

There are few quantitative nutritional requirements for crayfish. Huner and Meyers (1979) recommended 20–30% dietary crude protein for *P. clarkii*; crayfish were fed practical diets in that study. Hubbard et al. (1986) recommended a protein to energy ratio of 120 mg protein/kcal for *P. clarkii* fed purified diets. That ratio corresponded to 30% crude protein and 2.5 kcal gross energy/g diet. Davis and Robinson (1986) recommended a dietary lipid concentration of 6% or less for *P. acutus acutus*. Crayfish in the latter study were fed purified diets and menhaden oil was the source of dietary lipid. D'Abramo et al. (1985) recommended a dietary sterol concentration of 0.4% for *P. leniusculus*. Given this paucity of information, dietary formulation for crayfish is difficult.

### PRACTICAL DIETS AND FEEDING

Numerous species of crayfish are cultivated around the world. Thus, for each species, dietary development is in varying stages,

TABLE 3.  
Qualitative nutritional needs of freshwater crayfish.

Nutrient	Species	Reference
<b>Amino Acids</b>		
Arginine	<i>Astacus leptodactylus</i>	Zandee 1966
Lysine	..	..
Methionine	..	..
Tryptophan	..	..
Threonine	..	..
Phenylalanine	..	..
Leucine	..	..
Valine	..	..
Isoleucine	..	..
Histidine	..	..
Asparagine	..	Van Marrewijk and Zandee 1975
..	<i>Orconectes virilis</i>	Wetzel 1993
<b>Lipids</b>		
Cholesterol	<i>Astacus astacus</i>	Zandee 1966
Cholesterol or Phytosterols	<i>Pacifastacus leniusculus</i>	D'Abramo et al. 1985
Linoleic acid (18:2n-6)	<i>Astacus astacus</i>	Zandee 1966
Phosphatidylcholine	<i>Procambarus clarkii</i>	Lochmann et al. 1992
<b>Vitamins</b>		
Ascorbic acid	<i>Orconectes virilis</i>	Brown, unpubl. data

with most of that development in preliminary stages. The early studies with most species start with practical dietary formulations for fish or other crustaceans from the same geographic locale. Extrapolation of nutritional data from vertebrates (fish) to invertebrates is often the only possible option, but must be considered a poor formulation strategy. As outlined above, several nutritional requirements for arthropods are unique. Extrapolation from marine crustaceans, such as penaeid shrimp or homarid lobsters, to freshwater crayfish should be considered a closer approximation, but an approximation nonetheless. Despite our inability to precisely formulate a diet for crayfish, several research projects demonstrated the utility of nutritional inputs into culture systems for the production of crayfish.

### Diets

Provision of nutrients to aquatic animals is a function of the production system. For example, crayfish reared in tank culture systems do not have access to organisms that would be found in a pond culture system. Thus, crayfish reared in tanks must be fed diets that are as nutritionally complete as possible. For example, Reigh et al. (1993) evaluated soybean meal as a replacement for fish meal in diets fed to *P. clarkii* reared in tanks. Weight gain was highest in crayfish fed a 1:3 ratio of soybean meal to menhaden fish meal. Diets used in those studies contained 32% crude protein and 4.3–4.4 kcal gross energy/g diet; well above the recommended levels of Hubbard et al. (1986).

The contribution of organisms living in ponds to the nutrition of crayfish is significant (Huner 1994), so those reared in ponds can often be fed supplemental feeds. Diets formulated for fish could be considered supplemental for crayfish because they may not contain some of the unique nutrients required for crayfish at concentrations that support maximum weight gain.

Most of the early evaluations of feeding *P. clarkii* used diets formulated for warmwater species of fish because they were available and relatively inexpensive. Feeding those diets to crayfish reared in earthen ponds often resulted in increased production (Smitherman et al. 1967, de la Bretonne et al. 1969, Clark et al. 1975), but was not justified economically. Since those evaluations, supplemental feeds became simpler. For example, Lochmann et al. (1992) demonstrated the need for a supplemental vitamin premix in diets fed to *P. clarkii*, but could not demonstrate the need for supplemental minerals.

Feeds containing lower levels of crude protein (25%) (Huner 1994), range cubes (9–10% crude protein) (Cange et al. 1982), and crayfish baits (Huner 1994) have been used. Feeding all supplemental feeds increased production, but the economics were still questionable. More recently, corn grain, alfalfa pellets, rice bran, soybean meal, unprocessed soybeans and rice were evaluated as supplemental feeds for *P. clarkii* (Dr. J. V. Huner, pers. comm.). All products seem to have potential and not impair the economics of crayfish production in Louisiana. Similarly, Brown et al. (1995) were unable to demonstrate a beneficial effect of feeding near-complete feeds to *O. virilis* reared at high densities; feeding alfalfa hay and wheat straw resulted in comparable productions and growth. Given the relatively consistent finding that supplemental foods enhance production, additional research into feeds and feeding practices seems warranted. However, these findings also raise the question regarding nutritionally important foods in a pond setting.

### NATURAL FOODS

Crayfish eat almost anything they encounter in an aquatic environment. One of the early list of foods consumed by crayfish

included "worms, small mollusks, insects that fall in their way, small fish, and in general any kind of animal food especially carion" (King 1883). That list has been expanded in the twentieth century. Perhaps a more important question is which of those food items is nutritionally important; that is, which provide the complement of essential nutrients most closely matching the needs of crayfish and which are palatable or preferred foods.

There are 45–50 essential nutrients required by most animals. It is not practical to measure all nutrients in the potential food items of crayfish. Further, few nutritional laboratories are analytically equipped to measure all nutrients. Thus, growing crayfish by offering potential food items seems the only practical approach to distinguishing among natural foods.

Most species of crayfish are thought to consume plant material. Both macrophytes, forages and algae have been implicated (Budd et al. 1978, Huner and Barr 1984, Huner 1984). However, when adult *O. virilis* were offered 14 different aquatic macrophytes, overall mean consumption averaged 0.2% of body weight per day with a maximum consumption of 0.9% bw/day for crayfish fed a dried, pelleted duckweed (*Lemna* sp.) (Brown et al. 1990). Moshiri and Goldman (1969) reported dry matter digestibility values of 37–56% for an unidentified mixture of oven-dried macrophytes. Further, apparent dry matter digestibility ranged 25–40% for crayfish fed fresh or decomposed elodea (*Egera densa*) (Wiernicki 1984). The data developed thus far do not support the contention that fresh or decomposed macrophytes are nutritionally important foods for crayfish, although they may be sources of selected nutrients. The other argument was that crayfish were actually obtaining a significant portion of their nutritional needs from bacteria growing on macrophytes or planted forages. The strength of that argument has diminished with publication of some recent studies (McClain et al. 1992, Momot 1995). However, bacterial colonization and degradation of forages occurs in earthen pond systems and that decomposition changes the carbon:nitrogen ratio of the substrate. Specific C:N ratios have been recommended for better forage utilization by crayfish (Robinson 1989), although this is relatively difficult to control in earthen ponds. Discussions of recommended forages and cover crops can be found in Huner and Barr (1984, 1991), Robinson (1989), and Avault and Brunson (1990).

Brown et al. (1992) offered first feeding *P. clarkii* 3 potential food items in a simulated, yet controlled, pond environment. Weight gain was dramatically higher when crayfish were fed *Daphnia* than when offered a plant or unidentified pond bacteria grown in culture. Based on those data, it appears crayfish can utilize bacteria as a source of nutrients, but zooplankton appears a more important source of nutrients. The authors suggested managing zooplankton communities in ponds at the time juvenile crayfish begin exogenous feeding. Subsequent studies identified lysozyme (muramidase EC 3.2.1.17) activity in the stomach of crayfish that was not associated with ingested food items (unpubl. data). Lysozyme is one of the few enzymes capable of degrading bacterial cell walls. Thus, the generalization that crayfish consume bacterial colonies growing on decaying forages seems correct; however, other natural foods seem more important for growth.

### CONCLUSION

Crayfish nutritional research has not proceeded at a rapid rate, largely because the increased cost of feeding associated with the relatively low market price does not justify diet development for

crayfish reared in ponds. Crayfish reared in tanks need nutritionally complete feeds, but that remains a small segment of the world's crayfish production. In recent years, increased attention has been focused on production of large crayfish for selected markets and that change may stimulate nutritional research. In the

interim, crayfish can be produced at reasonable costs using inexpensive agricultural forages as feed inputs into earthen pond culture systems. Those inputs seem to be stimulating primary and secondary productivity that actually serve as the most important nutritional sources for crayfish.

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## AUSTRALIAN CRAYFISH CULTURE IN THE AMERICAS

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**ABSTRACT** Australian crayfish have generated the interest of aquaculturists in the Americas. Of the 5 commercially significant species in Australia, the red claw crayfish *Cherax quadricarinatus* is considered to have the greatest potential for commercial aquaculture outside Australia. Reproduction is easily accomplished in indoor tanks and culture ponds. Juveniles are released from the female so no larval rearing is required. In temperate climates juveniles are raised in indoor tanks for 1 to 2 months before stocking into ponds. In tropical countries, breeding and nursery rearing is accomplished in ponds. Culture ponds are usually stocked at densities of 4-6 m<sup>-2</sup> and juveniles can attain a market size (100 g) in 6-8 months. Feeding includes vegetative forages supplemented with pelleted crustacean diets. Yields of about 2,000 kg ha<sup>-1</sup> are reported after 6 months.

**KEY WORDS:** Australian crayfish, red claw, aquaculture

### INTRODUCTION

Crustaceans such as shrimp, prawns and lobsters are among the highest priced food items consumed. With the success of freshwater crayfish culture in the United States and marine shrimp culture in Latin America and Southeast Asia, it is no surprise that entrepreneurs have been attracted to several large species of crayfish from Australia. Advertisements appeared in aquaculture trade magazines in the mid-1980s claiming giant freshwater lobsters from Australia that could be grown to over 2 kg in backyard ponds. These enticing advertisements have stimulated considerable interest across the United States.

With the exception of North America, Australia has the largest and most diverse freshwater crayfish fauna in the world. Over 100 species of crayfish live in Australian rivers, lakes and swamps being variously referred to as crays, crawchies, juglies, koonacs, lobies, lobsters, marron or yabbies. All Australian crayfish belong to the family, Parastacidae, which includes 9 genera. Of all the endemic species, only five are harvested on a regular basis as part of a recreational fisheries (Merrick and Lambert 1991). Two genera of notable interest because of their large size are *Astacopsis* and *Euastacus*. *A. gouldi*, a species restricted to Tasmania, attains weights of up to 3 kg with unofficial records over 6 kg (Olszewski 1988). *E. armatus*, the Murray cray, is found in southeastern Australia and attains a weight of almost 3 kg (Sokol 1988). Both species are slow growing and have little potential for aquaculture. Aquaculture interest in Australia has been primarily centered on 3 species, *Cherax tenuimanus* or marron, *C. albidus-destroyer* or yabbie and *C. quadricarinatus* or red claw.

The first documented culture trials with Australian crayfish in the United States occurred in the early 1970s (Shireman 1973). A one-year study concluded that marron were not suitable for culture in the high summer water temperatures in Louisiana. After this effort, little if any interest was shown in these crayfish outside of Australia until the mid-1980s. About 1985, several private producers and universities began to investigate the different species with an interest in their potential for aquaculture. This paper reviews biological traits of marron, yabbie and red claw and the research and development effort that has taken place with them in the Americas during the past 10 years.

### TRAITS OF THE PRIMARY CULTURE SPECIES

Marron are native to southwestern Australia. They reach the largest maximum size (2,250-2,700 g) of the 3 primary species

but have an average annual growth rate of only 20 to 50 g year<sup>-1</sup> (Morrissey 1970). Marron are difficult to spawn under controlled conditions. They take 2 to 3 years to reach sexual maturity, are seasonal spawners, usually spawning in the spring as water temperatures and photoperiod increase (Morrissey 1970, 1976). They are cool water crayfish, surviving best at temperatures of 22 to 25°C and below (Morrissey 1976, Kartamulia and Rouse 1992). They are sensitive to water quality changes and handling and require a salinity of at least 100 mg L<sup>-1</sup> to molt successfully (Rouse and Kartamulia 1992). Marron are considered non-burrowing crayfish but are territorial.

Red claw are native to northern Australia. They are reported to reach a maximum size of 400 to 600 g (Hutchings 1987, Jones 1990) attaining 50 to 100 g in 6 months (Pinto 1994, Semple et al. 1995). Red claw reach sexual maturity at about 6 to 8 months of age and are considered multiple spawners, spawning 3 to 5 times a year (Sammy 1988, Jones 1990). Reproduction is easily obtained in tanks and ponds under culture conditions. Red claw are tolerant to a wide range of water quality conditions commonly occurring in culture operations. They survive water temperatures between 12 and 34°C but grow best within a range of 22 to 30°C. Red claw are reported to be non-burrowers (Hutchings 1987); however, shallow burrows have been reported (Medley et al. 1993).

Yabbie are native to southeastern Australia. They are reported to reach 140 to 280 g and commonly grow an average of 20 to 40 g year<sup>-1</sup> (Mills and McCloud 1983). Yabbie reach sexual maturity in about 6 to 8 months and may spawn 3-4 times in a season (Frost 1974, Johnson 1978). They tolerate temperatures from 7 to 34°C but grow best within a range of 22 to 30°C. Yabbie are considered to be territorial and capable of excavating deep burrows in pond dikes.

Red claw were considered to have the best traits for commercial aquaculture (Semple et al. 1995). At present, red claw are the only Australian crayfish being cultured in the Americas on a commercial scale.

### INDOOR HATCHERIES FOR RED CLAW

#### Spawning

In the tropics and subtropics of Australia, red claw breeding is normally accomplished in ponds. Because red claw will not survive winter conditions in temperate climates, indoor spawning is utilized. Initial trials indicated that mature red claw could be held

at relatively high densities in indoor tanks and would easily mate and produce juveniles. Research has been conducted at several institutions and private hatcheries to refine hatchery technology. Photoperiod and temperature were found to have a significant influence on spawning rates (Yeh and Rouse 1995). Spawning begins at about 20°C and increases to about 30°C. Average monthly spawning rates at 28°C ranged from 15% with less than 12 hr of light to over 35% with more than 12 hr of light. Peak spawning occurred with day lengths of 14 hr, but high spawning rates could only be maintained for about 3 months (Rouse and Yeh 1995).

Effects of stocking density and sex ratios on red claw reproduction in indoor tanks has been evaluated. Results showed no suppression of spawning at densities from 20 to 32 red claw  $m^{-2}$  (Medley 1994, Yeh and Rouse 1995). Male size did not affect spawning success when males were within 25 g of each other but mixed male sizes (small, medium and large, together) resulted in a 49% reduction in spawning. Female size had no effect on spawning activity (Medley 1994). Spawning rates were also similar at sex ratios of 1:1 to 1:5 M:F (Yeh and Rouse 1995).

Breeders accept a wide variety of feeds from fresh vegetable matter to chopped meats and manufactured diets. They are slow feeders usually grazing slowly over long periods of time. Varying dietary protein levels from 30 to 45% and adding beef liver or soybean supplements had no effect on numbers of eggs per gram of female but did decrease mortalities due to cannibalism among broodstock (Medley 1994).

After spawning, red claw carry their eggs attached to their pleopods until hatching. At 28°C, incubation required about 30 d (Yeh and Rouse 1994). Others have reported incubation lasting 40 to 70 d under different environmental conditions (Jones 1990, King 1993, Medley 1994). During incubation, changes in egg color appear to be consistent and predictable (Jones 1990, Yeh and Rouse 1994). Eggs are cream colored when first spawned, khaki or olive-green after 1 wk, dark brown after 2 wk, reddish after 3 wk and hatch after 4 wk. Juveniles are released 7 to 10 d after hatching. Some reports suggest that these color changes may vary slightly with red claw strain.

Fecundity is an important part of the measure of reproductive potential of a culture animal. It's usually defined as the number of eggs produced per spawn. Yeh and Rouse (1994) found that females with newly spawned eggs had an average of 10 eggs per gram of body weight. About 30% of the eggs were lost during incubation, resulting in an average of 7 eggs  $g^{-1}$  of female body weight at hatching. Broodstock held under stressful conditions and first time spawners usually have fewer eggs. One large red claw female (190 g) had 1,348 eggs resulting in the highest fecundity reported of any freshwater crayfish in the world (Yeh and Rouse 1994).

#### Juvenile Rearing

Spawning in indoor tanks has been relatively successful but rearing juveniles at high densities has been more difficult. Stocking densities from 50 to 1,250 juveniles  $m^{-2}$  have been reported. Survival rates ranged from 84 to 95% at densities up to 250  $m^{-2}$  but decreased rapidly at higher densities. Final juvenile weight was significantly higher at densities of 100  $m^{-2}$  and lower. There was no difference in final weight at densities of 250  $m^{-2}$  and greater (Yeh and Rouse in review). Window screen placed in a horizontal position as additional substrate improved survival, was cheaper and more practical than other substrates evaluated. Size

grading at 4 wk demonstrated a small but statistically significant increase in survival (Raisbeck 1994). At a density of 250  $m^{-2}$ , a feeding rate of 40% of the estimated biomass per day, resulted in the highest juvenile survival (64%) and weight (1.13 g) after 60 d (Yeh and Rouse, in review). Anson and Rouse (1995) evaluated commercially available diets and reported the best results (80% survival after 28 d) by feeding commercial shrimp pellets supplemented during the first week with *Artemia* nauplii. Webster et al. (1994) evaluated protein levels of 25–55% in practical feed formulations and reported that a diet containing 33% protein appeared adequate.

Red claw juveniles have the ability to regulate oxygen consumption rates over a wide range of environmental conditions yet do not utilize anaerobic pathways to withstand hypoxic or anoxic conditions (Meade 1995). Weight gain and survival occurred over the temperature range from 16 to 32°C but maximum weight gain and survival was observed at 28°C. Weight gain and survival was also observed over the salinity range from 0 to 20 ppt and was reduced at salinities above 5 ppt (Anson and Rouse 1994, Meade 1995).

Tolerance of juvenile red claw to ammonia, nitrite and nitrate are similar to those reported for other crayfish species. Rouse et al. (1995) reported red claw hatchlings had a 96 hr  $LC_{50}$  value of 1.03  $mg L^{-1}$  for nitrite toxicity while Liu (1994) reported a 96 hr  $LC_{50}$  value of 4.7  $mg L^{-1}$  for older juveniles. Studies on sublethal effects of ammonia and nitrites reveal that exposure of 1.7  $mg L^{-1}$  total ammonia or 0.4  $mg L^{-1}$  nitrite (28°C, pH 7) significantly reduced growth of juvenile red claw (Liu 1994, Rouse et al. 1995).

#### OUTDOOR CULTURE IN TEMPERATE CLIMATES

A water temperature of about 20°C is considered safe to move red claw to outdoor ponds. Culture ponds generally range from small ponds of 0.1 ha up to 1 ha but are generally 0.25 to 0.5 ha in size. Depths generally range from 0.7 to 1.0 m. Red claw, like most crayfish, are detritivores and opportunistic carnivores, and feed on most types of organic matter (Jones 1990). Feeding strategies have combined forage base systems typical of those used for North American red swamp crayfish with pelleted rations commonly used for other crustaceans. Red claw are cultured in warm water so much lower amounts of forage are used to prevent low dissolved oxygen. Dry hay added at about 500  $kg ha^{-1}$  per month divided into 2 to 3 applications has been used effectively. Pelleted shrimp or crayfish rations are also fed at about 3% of the estimated biomass per day (Pinto 1994, Kahn 1995).

Potential ecological impacts from the introduction of red claw were considered. Experiments were conducted in year-round culture conditions to determine potential interactions on red swamp crayfish (Medley et al. 1993). General health of red claw and red swamp crayfish were monitored throughout the year as the 2 species were cultured together and separately. No significant disease outbreaks occurred. Australian red claw grew well from May through October but did not survive the winter. Red swamp survived and grew well only during the cooler months. No negative interactions on red swamp were observed (Medley et al. 1993).

Pinto and Rouse (in press) considered effects of red claw stocking densities when cultured during a 5-month growing season. Survival rates (72%) did not vary but final weights and yields differed significantly. When stocked at 10,000 juveniles  $ha^{-1}$ , red claw averaged 67 g with a total yield of 475  $kg ha^{-1}$ . At 30,000  $ha^{-1}$ , average weight was 48 g with a yield of 1,020  $kg ha^{-1}$  and at 50,000  $ha^{-1}$ , the average weight was 38 g with a yield of 1,422

kg ha<sup>-1</sup>. An economic evaluation of these results indicated that commercial potential for red claw culture is most sensitive to the cost of juveniles, percentage of harvestable biomass in the large size classes, price these classes receive and the length of the growing season (Medley et al. 1994).

To increase yields and profits, red claw polyculture has been evaluated. To date, all red claw polyculture experiments have been conducted with tilapia. In Israel, Karplus et al. (1995) attributed reduced red claw yields to predation by the African catfish, *Clarius gariepinus* and the freshwater crab, *Potamon potamios* that accidentally entered red claw-tilapia ponds. In the United States, Brummett and Alon (1994) and Kahn (1995) experienced similar reduction in red claw yields without unwanted predators. These early experiences suggest that a red claw-tilapia polyculture may not be beneficial. Competition with the tilapia for food and space and the impact of reduced water quality appear to be the primary factors affecting red claw growth (Kahn and Rouse, in press).

### RED CLAW CULTURE IN TROPICAL COUNTRIES

Red claw farming began in tropical regions of the Americas about 1993. Farms now occur in Central and South America and the Caribbean. These farms range in size from a few hectares to several farms with almost 50 ha of ponds. Most farms are utilizing pond spawning techniques similar to those developed in Australia combined with large-scale growout.

Spawning and nursery operations are performed in small ponds (0.1 to 0.25 ha). Ponds are stocked with mature red claw at densities of about 2 m<sup>-2</sup>. Spawning usually begins within a few months after stocking. Mesh-bag collectors are used to trap juveniles of 3 to 6 g from the spawning-nursery ponds. Bag material (5 to 10 mm mesh) like that used for holding onions and potatoes are gathered into bundles, weighted on one corner with a rock and placed in about 0.5 m of water. Juvenile red claw seek shelter in the folds and graze natural foods such as algae and microcrustaceans growing on the mesh. Juveniles are harvested by gently lifting the folded bag until a fine-mesh dip net can be slipped under the collector and the juveniles shaken off. Traps are usually placed about every 3 m around the pond's edge and checked at 2 to 3 day intervals. Estimates vary widely but juvenile collections may run

between 500,000 and 1,000,000 juveniles ha<sup>-1</sup> every 4 to 5 months.

Some farm managers stock directly into 0.5 to 1.0 ha growout ponds at densities of 30,000 to 70,000 juveniles ha<sup>-1</sup> while others stock higher densities into intermediate ponds for about 3 months and then harvest and restock at lower densities for another 3–4 months. Feeding strategies are similar to those used in temperate climates. Locally available forage materials such as dry hay or corn silage are provided as a food supply. Forages are then supplemented with daily applications of pelleted crustacean rations at 2–3% of the estimated red claw biomass. Water exchange is usually held to a minimum with enough water to replace evaporation and seepage. Some farms have begun to use mechanical aerators to maintain satisfactory levels of dissolved oxygen and to increase water circulation.

Harvesting is accomplished with flow traps. Red claw are attracted by flowing water so fresh water is pumped from a nearby supply canal or pond into a box which overflows through an enclosed ramp positioned between the top of the box and the pond bottom. Red claw move up the ramp following the flow and fall into the box from which they can't escape. With good techniques some producers report 90% of the crop may be harvested from a production pond during a single night.

Most producers expect about 1,500 to 2,000 kg ha<sup>-1</sup> per crop with 2 crops a year when 80 to 120 g red claw are produced. Yields can be increased with higher densities but harvest size usually decreases as density increases.

### SUMMARY

The red claw crayfish from Australia appears to possess many traits desirable of a good aquaculture species. It tolerates a wide range of environmental conditions, is easily reproduced in captivity, and grows to market size in about 6 months. Indoor hatchery technology has been developed for use in temperate climates but production costs are high. Pond spawning in tropical countries allows for inexpensive production of juveniles which could provide needed juveniles for growout in both temperate and tropical regions. Technology seems to be adequate where climate allows for a 6-month growout.

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**ABSTRACTS OF TECHNICAL PAPERS**

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**CAUSES AND IMPACT OF MORTALITIES IN CULTURED SHELLFISH ON THE PACIFIC COAST OF NORTH AMERICA.** S. M. Bower\*, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada, V9R 5K6.

Although significant mortalities are not often encountered, the potential for substantial losses exists and is manifested on occasion. For all juvenile bivalves, hinge ligament disease caused by ubiquitous *Cytophaga* bacteria that erode hinge material are of concern especially if the bivalves are less than 1 cm in shell length and experience a stress that temporarily limits growth. Numerous flatworms, *Pseudostylochus ostreophagus* (Class Turbellaria), have caused high mortalities (up to 100%) among juvenile Japanese scallops (about 1 cm in shell height) and have the potential of being equally harmful to juvenile mussels and oysters. Market-size Pacific oysters can have unsightly green lesions and up to 30% mortality attributable either to nocardiosis caused by bacteria in the genus *Nocardia* after warm summers or to Denman Island disease caused by the protozoan *Mikrocytos mackini* after 3 to 4 months at about 10°C. Other species of oysters seem more susceptible to the pathogenic affects of *M. mackini* and may also be vulnerable to nocardiosis. To date, Manila clams in the eastern Pacific are not known to experience losses from infectious diseases. However, in northern areas, winter low tides that are concurrent with freezing temperatures and surface winds have resulted in high mortalities that do not become apparent until spring. Apart from duck predation, which can be devastating, 100% of intensively cultured blue mussels can have haemocytic neoplasia, and annual cumulative mortalities may exceed 75%. However, some blue mussel species and/or stocks appear resistant to this disease. Japanese scallops in a few grow-out localities occasionally experience high mortalities (>90%) attributable to a new protozoan called SPX. Cultured geoducks run the risk of developing unsightly neck scars of unknown etiology that occur on some wild individuals. Understanding the cause of mortalities that affect cultured shellfish will undoubtedly lead to management tools for prevention or avoiding serious losses.

**WESTERN REGION AQUACULTURE INDUSTRY SITUATION AND OUTLOOK REPORT: A SHELLFISH PERSPECTIVE.** Kenneth K. Chew, Western Region Aquaculture Center, School of Fisheries Box 357980, University of Washington, Seattle, WA 98195-7980; Derrick R. Toba\*, Western Region Aquaculture Center, School of Fisheries Box 357980, University of Washington, Seattle, WA 98195-7980.

The Western Region Aquaculture Center (WRAC) conducted a survey to estimate the aquaculture production in the twelve western states: Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. Information on finfish (salmon, trout, catfish, tilapia, and

others), shellfish (oysters, mussels, and others), aquatic plants, and non-foodfish were collected in the survey.

Total aquaculture for the western region was 158,000 pounds and valued at \$276,000. Of the total, 86,000 pounds (54%) and \$46,000 (17%) were through shellfish. Five states reported commercial shellfish aquaculture harvests and sales: Alaska, Arizona, California, Oregon, and Washington. In each of the coastal states, oysters comprised the majority of the shellfish production. Other species harvested include mussels, clams, scallops, abalone, crawfish, and shrimp. Trends over the past five years are indicated for each state and species.

**IS SHELLFISH FARMING IN THE PACIFIC NORTHWEST LIVING UP TO ITS POTENTIAL?—A CANADIAN PERSPECTIVE!** W. E. Lorne Clayton, RPBio., Gigas Growth Systems Inc., 4829 Maple Grove Street, Victoria, British Columbia, Canada, V8Y 3B9.

Many predictions have been made over the years of the economic potential of shellfish aquaculture in the Pacific North West. Accordingly there are mixed opinions on whether this industry has already reached its maximum potential, never will, or is only just beginning to develop.

This paper intends to give one grower's perspective on the status of this industry, the opportunities which lay ahead and the constraints against which the industry must struggle to prove itself. Topics for discussion include: status of the industry; public perception; regulatory constraints; resource availability; current technology; investor perception; product development and, marketing.

**DIVERSIFICATION OF SHELLFISH HATCHERY PRODUCTS.** Jim Donaldson, Hatchery Manager, Coast Seafoods Company, P.O. Box 327, Quilcene, WA 98376.

Hatcheries have achieved tremendous success over the last 20 years in supplying the basic needs of the shellfish industry of the west coast of North America. Pacific oysters and Manila clams provided the basis for development of hatcheries in the early 70's. The past and current level of hatchery production of these two species is described, as well as the past and current prices of these products.

The basic hatchery techniques in use today are described for production of algae with continuous or batch culture, larval rearing with static tank culture and continuous culture, setting on cultch or cultchless, and nursery culture in upwell systems or beach nurseries.

New hatchery species and products are described which enable hatcheries to diversify their products and sales, and to continue meeting the needs of the shellfish industry. These include species already in production such as Kumamoto and triploid oysters, Gallo mussels, and Geoduck clams, and potential candidates for culture such as larval fish and Abalone.

**USING INTERTIDAL SHELL PLACEMENT TO DISCOURAGE BURROWING SHRIMP INVASION IN OYSTER BEDS: WILL IT WORK AND WHY?** Kristine L. Feldman\*, David A. Armstrong. School of Fisheries, P.O. Box 357980, University of Washington, Seattle, Washington 98195; Brett R. Dumbauld, Washington State Dept. of Fish and Wildlife, P.O. Box 190, Ocean Park, Washington 98640.

A field experiment was initiated in July 1994 to examine whether the addition of oyster shell to carbaryl-treated and untreated mudflat discourages recruitment success of young-of-the-year (YOY) burrowing shrimp, *Neotrypaea californiensis*, while providing a substrate suitable for oyster culture. Large (1000m<sup>2</sup>) and small plots (64 m<sup>2</sup>) were constructed at Nahcotta, Willapa Bay, Washington, and assigned to one of four treatments: carbaryl + shell (CS), carbaryl (C), shell (S), and untreated mud (M). Oyster seed was planted on plots the following spring (April 1995). YOY shrimp densities, percent epibenthic shell cover, and seed densities were monitored from July 1994 through August 1995.

Only carbaryl had a negative effect on YOY shrimp recruitment, although epibenthic shell cover was significantly higher on carbaryl-treated plots (CS) than untreated plots (S). Trends in shrimp densities were similar for large and small plots: recruitment was highest into untreated mud plots (M), intermediate in untreated shell plots (S) and lowest in carbaryl (C) and carbaryl-treated shell plots (CS). In June 1995, carbaryl-treated shell plots (CS), still largely devoid of *Neotrypaea californiensis*, were colonized by the mud shrimp, *Upogebia pugettensis*. Oyster seed, planted initially at a density of 10 shells m<sup>-2</sup> on small plots sank rapidly on non-carbaryl-sprayed plots (S and M) to <1 shell m<sup>-2</sup> within 2 months. Seed densities on carbaryl-treated plots (C and CS), in contrast, have stabilized at approximately 6 shells m<sup>-2</sup>. Plots will continue to be monitored through June 1996 to assess further changes.

**FUTURE OF FLOATING CULTURE IN BRITISH COLUMBIA.** Norm Gibbons\*, Redonda Sea Farms Ltd., Box 89, Mansons Landing, British Columbia, Canada, V0P 1K0.

Evidence is presented which suggests that floating culture as proposed by Dr. Dan Quayle as long ago as 1969, can exceed his production capability projections.

The convergence of new technologies at every level, including genetic research, hatchery developments, nursery improvements, grow-out equipment, harvesting capabilities, processing improvements and new markets and products; make it possible to attain yields previously thought impossible. In addition, the wealth of experience, knowledge and expertise of present industry members, set the stage for dramatic increases in oyster production and short cuts to development of new species for culture.

Biological and regulatory impediments to this positive outlook will also be discussed.

**DISTRIBUTION AND BIOLOGY OF THE EXOTIC VARNISH CLAM, *NUTTALLIA OBSCURATA* (REEVE, 1857), IN THE STRAIT OF GEORGIA, BRITISH COLUMBIA.** Graham E. Gillespie\*, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6.

A new clam found in British Columbia waters was identified in 1993 as *Nuttallia obscurata*, a Japanese species. It was recently introduced into the Strait of Georgia, possibly as larvae in ballast water. The clams are oval, with compressed valves. The hinge ligament is external and large. The periostracum is thick and shiny brown. The interior of the valves is a uniform purple. The mantle, siphons and body are white. The siphons are very long, and split to the base.

Varnish clams were recorded from Manson's Lagoon, Cortes Island (50°05'N, 125°00'W) in the north to Boundary Bay (49°00'N, 122°45'W) and Victoria (approximately 48°25'N, 123°20'W) in the south. They were found mainly on low-slope beaches with sand, gravel and mud substrates. They were found at all levels of the beach, at times higher than Manila clams. They have good tolerance of freshwater, and were often found in or near stream channels. They were buried up to 20 cm in the substrate.

The effect of this introduced species on other clam populations is yet to be determined. While they are found in low densities in association with Manila and littleneck clams, their greatest densities occur in under-utilized habitats. One individual harbored a pea crab, *Pinnixia faba*. Numerous shells showed evidence of predation by moon snails, *Polinices lewisi*. Gulls ignored varnish clams, even when opened and offered to them.

Edibility and potential marketability have not been established for this species.

**INCREASING PACIFIC COAST OYSTER PRODUCTION THROUGH SELECTION: LET'S DO IT!** Dennis Hedgecock\*, Bodega Marine Laboratory, University of California, Davis, Bodega Bay, CA 94923-0247.

The genetic gains in agricultural production that have been achieved by selection are well known. In aquaculture, selection programs for salmonids, channel catfish, and tilapia have documented genetic gains of 10-12% per generation for growth rate. Substantial increases in resistance to particular diseases have also resulted from challenge experiments with Atlantic salmon. Previous research with the Pacific oyster and other bivalves suggests that similar genetic gains would result from well designed selection programs.

The selection program for the Norwegian salmon-farming industry provides an excellent model for the Pacific coast oyster industry. Initiated in 1972 with government funding, this program progressed, from an initial phase of screening natural stocks, to the establishment of a closed brood stock population by 1980, to the fifth selected generation at present. After genetic parameters were estimated and selection methods designed, the Norwegian salmon

breeding program involved private companies as test sites for comparing families and as multipliers of select families. The multipliers practice mass selection to effect further genetic gains and produce fertilized eggs for fry and smolt producers, who in turn sell select fry and smolts to farmers. Convinced of the economic gains of this selection program, the Norwegian salmon industry collectively funded the construction of a second breeding station in 1986.

The recently funded Molluscan Broodstock Program (MBP) at the Hatfield Marine Science Center, Newport, OR, provides the vehicle necessary for initiating a selection program patterned after the highly successful Norwegian salmon breeding program. The MBP can provide, for the first time, a scientifically sound selection program based on sufficient numbers of pedigreed families. Industry cooperators will be needed for testing sites and multiplying stations. All indications are that this program can be successful in increasing production of Pacific oysters for generations to come.

**ASSESSMENT OF CARRYING CAPACITY OF GROWING AREAS AS A METHOD TO INCREASE SHELLFISH PRODUCTION ON THE WEST COAST OF NORTH AMERICA.** B. C. Kingzett, Kingzett Professional Services, P.O. Box 116, Errington, B.C. V0R 1V0; N. F. Bourne\*, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

Increased worldwide demand for seafood products provides a major incentive to increase shellfish production on the west coast of North America. However, it appears unlikely that governments will allocate any significant increases in growing areas to the shellfish industry in the immediate future, at least from California to central British Columbia, that would enable them to increase production. The most expedient way to attain increased production will be to insure maximum use is made of existing culture areas, as has been accomplished in agriculture. Achieving the goal of maximum production from existing areas will require knowledge of the carrying capacity of growing areas. Such information could lead to increased production per unit area. If carrying capacity is exceeded it can have an adverse effect and actually lead to decreases in production, as was observed from overcrowding of oyster rafts in the Inland Sea area of Japan. The present paper examines methods used to determine carrying capacity of bivalves in various world locations and equates them with conditions on the Pacific coast. Carrying capacity can be complicated and difficult to assess and it can also be site specific and vary considerably from year to year. However, it does provide a useful method to assist the shellfish farmer to obtain maximum production from culture areas.

**EXPERIMENTAL GROWOUT OF F<sub>1</sub> PACIFIC OYSTERS, CRASSOSTREA GIGAS, FROM TASMANIA IN WASHINGTON STATE WATERS.** Manfred T. Kittel\*, Kenneth K. Chew. School of Fisheries 357980, University of Washington, Seattle, Washington 98195.

One hundred and sixty adult Pacific oysters, *Crassostrea gi-*

*gas*, were imported into Washington state from Tasmania in compliance with regulations and requirements of the Washington Department of Fish and Wildlife (WDFW). All were held in quarantine at Battelle Laboratories in Sequim, Washington. Histopathological examination showed no evidence of any known infectious diseases in 60 of the Tasmanian *C. gigas* and 35 Olympia oysters, *Ostrea lurida*, that were co-cultured with 20 Tasmanian oysters for 60 days. The remaining live Tasmanian *C. gigas* were artificially spawned on two separate occasions in July, 1995. Larvae of the resulting F<sub>1</sub> generation were reared at the Taylor United shellfish hatchery in Quilcene, Washington. Seed oysters were planted intertidally and in suspension culture at different locations of Puget Sound and San Juan Island. Preliminary data suggest that the Tasmanian F<sub>1</sub> oysters grow as fast as local Miyagi *C. gigas* under hatchery conditions. Further studies will examine shell morphology, growth rate, gonadal development and other biological parameters of these Tasmanian oysters under local environmental conditions.

**MANILA CLAM AQUACULTURE ON SHRIMP INFESTED MUDFLATS.** Matt D. Smith\*, C. J. Langdon. Department of Fisheries and Wildlife, Hatfield Marine Science Center, Newport, Oregon 97365.

Hatchery seed (7.4 mm) of the Manila clam (*Venerupis japonica*) was planted on a mud shrimp-infested (*Upogebia pugettensis*) intertidal area in Yaquina Bay, Oregon. Substrate modification, and predator exclusion devices were used in combination to grow seed for 13 months. Predator exclusion devices were mandatory for adequate clam survival in the field, with in-ground cages providing superior protection compared to Vexar® netting (both were ¼ inch mesh size). The initial application of oyster shell to the substratum significantly affected recruitment and survival of young of the year (YOY) mud shrimp, as well as increased the concentration of shore crabs on test plots for the duration of the study.

Survival of clam seed was poor in unprotected and Vexar netted treatments, although caged treatments showed greater than 95% survival. Growth rates were similar in all treatments (2 mm/month during summer). Adult Hairy shore crabs (*Hemigrapsus oregonensis*) appeared to be the most serious predators of Manila clam seed.

Laboratory predator-prey studies were conducted with both juvenile Dungeness (*Cancer magister*—sizes post larval 2, 3, and 4) and adult Hairy shore crabs [size 17–22 mm carapace width (CW)]. Juvenile Dungeness crabs were more effective predators, consuming 1.5 to 2.5 times more Manila clams than adult Hairy shore crabs [daily consumption rates for 17–22 mm CW crabs: Dungeness—8.4 (9–12 mm) clams/crab, 26 (5–8 mm) clams/crab, 133 (2–4 mm) clams/crab; Hairy shore crab—3.3 (9–12 mm) clams/crab, 11 (5–8 mm) clams/crab, 92 (2–4 mm) clams/crab]. A size refuge of 17–20 mm was evident for Manila clams when being preyed upon by large (22 mm CW) Hairy shore crabs.

**FACTORS GOVERNING THE DISTRIBUTION, ABUNDANCE, GROWTH AND REPRODUCTION OF THE FRESHWATER MUSSEL, *MARGARITIFERA FALCATA*, IN FORESTED WATERSHEDS OF WESTERN WASHINGTON.** Kelly Toy\*, Washington Cooperative Fish and Wildlife Research Unit, School of Fisheries, Box 357980, University of Washington, Seattle, WA 98189-7980.

*Margaritifera falcata* (family: Margaritiferidae) is the most common species found in forested watersheds in western Washington. Currently there are no existing studies or population estimates for this species in western Washington. A survey was conducted in Battle Creek, located on the Tulalip reservation and Bear Creek, near Woodinville, Washington. Population size, growth rate, and age structure was determined for both streams. Mussel densities ranged from 55 to 84 mussels per m<sup>2</sup>. Bear Creek exhibits an older population and a low level of recruitment. The Battle Creek population has a greater variation in age structure than Bear Creek. Characterization of mussel habitat include substrate, current velocity, and water chemistry analysis. Samples will be collected to identify gametogenic stages, mode of reproduction, and time of glochidia release. A correlation between increase water temperature and glochidial release will be determined using temperature recordings over a one year period.

**A FISH CONSUMPTION SURVEY OF THE TULALIP AND SQUAXIN ISLAND TRIBES OF PUGET SOUND.** Kelly A. Toy\*, Gillian D. Gawne-Mittelstaedt. Tulalip Tribes, Natural

Resources Department, 7615 Totem Beach Road, Marysville, WA 98271; Nayak L. Polissar, Shiquan Liao. Statistics & Epidemiology Research Corporation, 1107 NE 45th Street, Suite 520, Seattle, WA 98105.

A survey was conducted to determine the fish consumption rates of the Tulalip and Squaxin Island tribes of Puget Sound. A total of 263 tribal members, aged 18 and older, were surveyed. Data were collected for 77 children between ages 0 and 5 years. Information was obtained for species consumed, parts of fish eaten, preparation methods, sources of fish and children's consumption rates. Weight-adjusted consumption rates were calculated by tribe, age, sex, income, and species group. Species groups (anadromous, bottom, pelagic, and shellfish) were defined by life history and distribution in the water column. Both median and mean fish consumption rate for adults and children within each tribe were calculated in terms of grams per kilogram body weight per day (g/kg/day).

The median shellfish consumption rate for Squaxin Island tribe was .065 g/kg/day (mean = .181). In the Tulalip tribe, the median shellfish consumption rate was .218 g/kg/day (mean = .423). The average body weight for Squaxin (82 kg) and Tulalip (81 kg) was calculated to present consumption rates in grams per day. The median consumption rate was 5.33 grams per day (mean = 15.0) in the Squaxin Island tribe. Median consumption rate was 18.0 grams per day (mean = 34.0) in the Tulalip tribe. The majority of shellfish consumed (62 to 72%) by both tribes was from inside the Puget Sound.

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**COVER PHOTO:** The giant clam *Tridacna gigas* at low tide in intertidal culture. (Photo courtesy of S. S. Mingoa-Licuanan; see article pp. 000-000)

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