



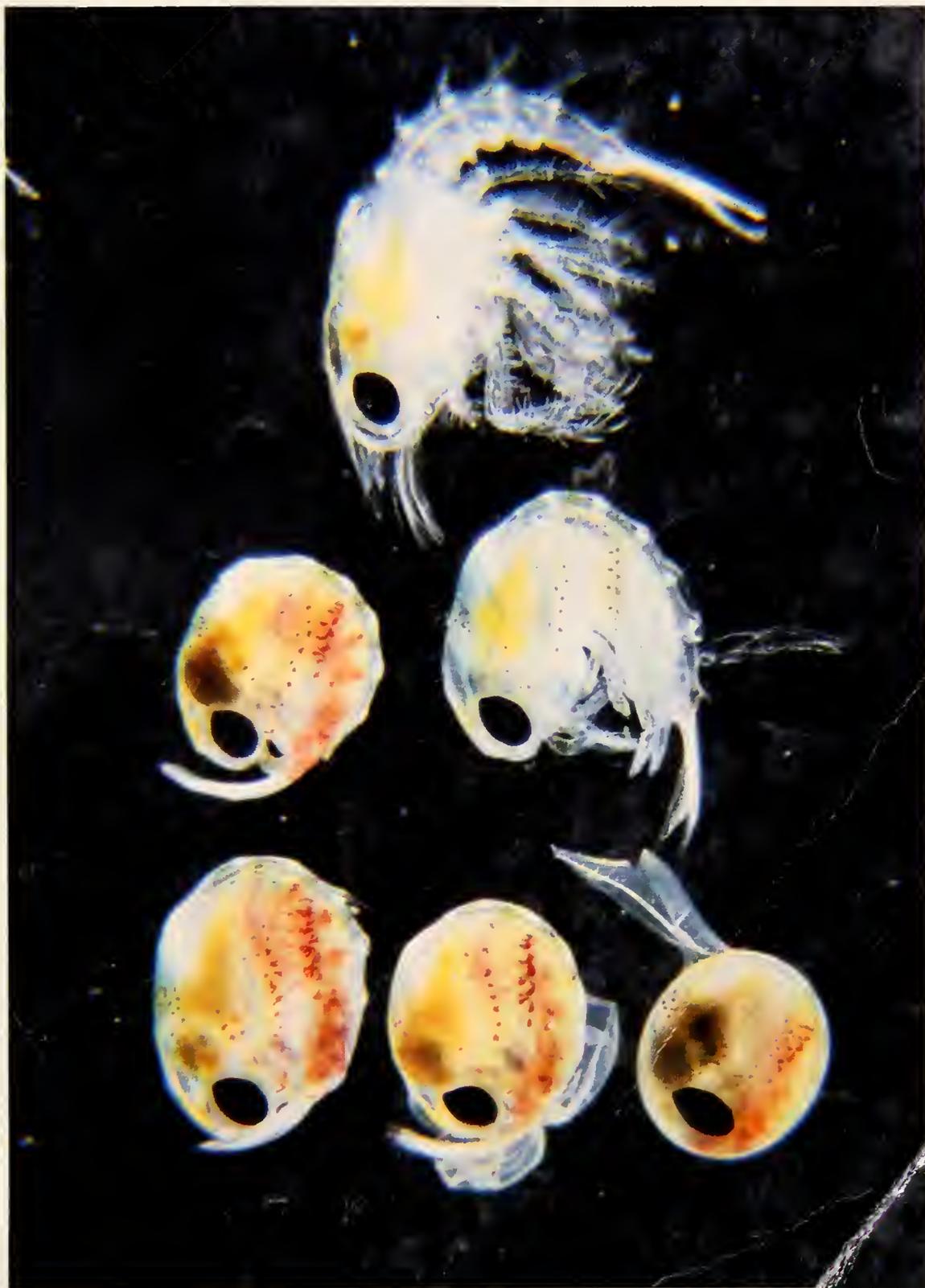




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**WARREN F. RATHJEN**  
**1929–1990**

Warren F. Rathjen died of a heart attack on 2 November, 1990 in Melbourne, Florida after a five-month illness. Warren Rathjen was known to cephalopod biologists as an enthusiastic and sagacious proselyte for the development of cephalopod fisheries, especially for squids. But, long before Warren became hooked on squids, he was known to a generation of fishery biologists as a hard-working, hands-on, classical fishery biologist, particularly on a variety of marine fin fishes. He knew the on-board sight, feel and smell of fishes, where they lived, how to catch them and how to process and market them. He was not particularly enthusiastic about the desk-bound, number-crunching type of fishery biology, and he was able to avoid it, much to the benefit of the field.

Born in New York City on 4 March, 1929, Warren Rathjen was exposed throughout childhood to the Long Island fishing industry and the outdoors. Upon graduation from high school in 1946, Warren served for three years with the U.S. Coast Guard in the western North Atlantic and the eastern North Pacific. He earned a Bachelor of Science in Zoology at the University of Miami (Florida) in 1953 and pursued graduate studies in fisheries in 1953 and 1954 at the University of Miami and the University of Washington. Warren took additional graduate work, in political science, at the George Washington University in Washington, D.C. in 1958–59.

Warren Rathjen served as a hydrographic observer at the Woods Hole Oceanographic Institute in 1953, then became an aquatic biologist for the state of New York (1954–56), working on the life history of striped bass and publishing his first two papers. Warren began his distinguished service with the National Marine Fisheries Service (then called the Bureau of Commercial Fisheries) in 1956 as a fishery methods and equipment specialist. He conducted exploratory fishing surveys for tuna, red snapper and shrimp in the Gulf of Mexico, Caribbean Sea, South America and Florida east coast from the laboratories in Pascagoula, Mississippi and Jacksonville, Florida. Then followed exploration and gear research on tunas and shrimp as Assistant Base Director (Exploratory Fishing and Gear Research) at Gloucester, Massachusetts (1959–62). During this period Warren published eleven more papers on various aspects of the fisheries and biology of fishes and shrimps.



W. F. Rathjen with deep-sea squid.

As Director of the Exploratory Fishing Base in Juneau, Alaska from 1962–64, Rathjen conducted exploratory studies on groundfishes, shrimps, crabs and scallops. He experienced another extreme change in habitat in 1964 when he went to Barbados as the Chief of Exploratory Fishing for the Caribbean Fisheries Development Project of FAO, United Nations. During a 5-year period he oversaw the planning and building of three fishery research vessels and headed research projects and exploratory fishing in 14 Caribbean territories and in waters off Central and South America. In addition to papers on fisheries topics, Warren also published several reports on sea turtles, whales and whaling, and even on human fatalities from stingray “stings.”

Rathjen migrated northward again to the NMFS Laboratory in Woods Hole in 1969 where he began fisheries surveys on underutilized species in the western North Atlantic. It was during this period that Warren became thoroughly captivated with squids as an underutilized fishery resource of potentially considerable importance to U.S. fishermen. Warren dedicated the rest of his professional career to studying the squid fisheries and potential, promoting the development of squid fisheries, marketing strategies, and handling technologies. Rathjen’s last position with the NMFS (1973–85) was as Fisheries Administrator, Deputy Division Chief, for fisheries development for the 19 states in the New England, Middle Atlantic and Great Lakes region. While Warren continued to encourage the development of fisheries for underutilized species, particularly red crab, ocean quahog, herring, mackerel and butterfish, his primary passion was squid fishery development and capture methods. All but four of his last 22 publications (1973–91) concerned squid fisheries, fishing techniques and gear. Rathjen published a total of 52 papers during his career.

After 32 years of government service, Warren Rathjen retired from the NMFS in the spring of 1985 and moved to Florida where he became Adjunct Professor at Florida Institute of Technology in Melbourne, working on fisheries gear and development. From 1987–89 Warren served as Marine Advisor for the University of Florida Sea Grant Program, working with the public on issues concerned with fisheries, environment, coastal processes and education. Since 1988 Warren was a consultant on squid and tuna fisheries development to the international fisheries company, Transpac Fisheries, Ltd.

Warren was appointed a certified Fishery Scientist of the American Fisheries Society, a Fellow of the American Institute of Fishery Research, and an Executive Member of the Cephalopod International Advisory Council. Rathjen also held lectureships at various times in Barbados, Massachusetts Maritime Academy, Salem State College, Yale University School of Organization and Management, and Florida Institute of Technology.

These facts about Warren Rathjen's distinguished career tell little about his character and nature. Warren certainly was a good-natured man with a wry sense of humor; his wit was much appreciated by his colleagues, especially when it was directed toward bureaucracy and desk-bound fishery science. He had a gentle, self-deprecating charm that enabled him to move as easily amongst fishermen and fishing industry representatives, the general public, students, and government officials, as among his peers. I doubt that Warren Rathjen ever failed to respond to a request for information; he was incredibly helpful to everyone and responded with the information requested, as well as with references to papers, and names and addresses of appropriate people in the field.

Warren's interests were broad, always associated in some way with the outdoors. He especially enjoyed sailing and gardening. He was an avid photographer, particularly of fisheries gear and lighthouses, which were a favorite subject, (a number of his lighthouse photographs were published, principally in *Sea Frontiers*). Warren also pursued an interest in the history of fisheries, fishing boats and gear, and fishermen. He liked nothing better than to spin salty yarns ("swap lies") with fishermen and sea-going fishery biologists. Finally, Warren was dedicated to conservation, and he served on the Gloucester (MA) Conservation Commission.

The community of cephalopod researchers and squid fishery representatives and, indeed the broader range of marine fisheries biologists, have lost too soon a productive and valued colleague and a loyal friend. But, having known him, we have gained ever so much. Our condolences are expressed to those who lost the most, Warren's wife, Helga, and their children, Diana and Lars, of whom he was very proud.

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## PLOIDY MANIPULATION IN MOLLUSCAN SHELLFISH: A REVIEW

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**KEY WORDS:** triploidy, tetraploidy molluscs, gynogenesis, aquaculture

### INTRODUCTION

Like most higher animals and plants, commercially important molluscs are diploid: each cell of the organism contains a matching set of chromosomes. For example, oysters have a diploid number (2N) of 20 chromosomes (10 pairs; haploid number, N = 10), mussels have 2N = 28 (14 pairs; N = 14) and most scallops have 2N = 38 (19 pairs; N = 19).

Normal cell division, mitosis, involves the complete replication and division of each chromosome into two daughter chromatids which pass, one each, into the daughter cells. During the process of sexual reproduction, however, the germ cells undergo two special and characteristic "maturation" divisions before becoming gametes (Fig. 1a-d). During the early stages of the first meiotic division (meiosis I), homologous chromosomes associate together to form a tetrad of four chromatids. Genetic recombination takes place as a result of crossing-over between non-sister chromatids of the tetrads. Halving of the chromosome number occurs at the end of meiosis I, when one chromosome from each homologous pair goes to each daughter cell. The second maturation division, meiosis II, follows immediately and is essentially similar to mitosis, in that each daughter cell receives one chromatid. Sexual reproduction is completed by syngamy, the uniting of a male and a female gamete to form the zygote.

Each male germ cell will produce four gametes after meiosis I and II are completed. Female germ cells, although following the same chromosomal pattern, divide unequally in meiosis I, such that one cell contains almost all the cytoplasm and the other contains almost none. This latter cell is the first polar body. Meiosis II follows a similar pattern to produce the final oocyte and a second polar body. Occasionally, in some species, the first polar body may undergo a meiosis II division, but neither polar body contributes any chromosomes to the zygote.

Although normal sexual reproduction involves both meiosis and syngamy, a number of variations of the process have evolved in certain groups (see Bell 1982, for review). Groups of organisms in which meiosis occurs but syngamy does not, are often loosely termed "parthenogens." In these organisms, diploidy is restored by allowing chromo-

somal replication but suppressing cell division either before (by endomitosis), during (at meiosis I or meiosis II) or after the maturation division (at first cleavage) (Bell 1982). Diploid gynogenesis is a special case of parthenogenesis, in which diploid eggs are produced, but sperm contact (egg "activation") is required to trigger the maturation divisions. The sperm does not participate in syngamy.

The process of "chromosome-doubling" (allowing chromosomal replication but suppressing cell division, Bell 1982), which has evolved naturally in parthenogens, provides the basis for artificial ploidy manipulation in fish and shellfish. In essence, triploids (3N) can be produced by suppressing meiosis I or II but allowing syngamy, and tetraploids (4N) by the suppression of first cleavage. The use of ultra-violet light treated sperm (which allows egg activation but prevents syngamy) together with suppression of either meiosis I, meiosis II or first cleavage, can produce diploid gynogenomes.

The importance of triploidy stems principally from the presumption that homologous chromosomes in the germ cells of adult triploids cannot synapse at meiosis and triploids are, therefore, expected to be sterile. From a commercial point of view, sterility is desirable for three main reasons. First, energy usually diverted to gamete production is available for somatic growth in sterile triploid individuals. Therefore, adult triploids should grow faster. For example, Davis (1988b) calculated energy budgets for diploid and triploid Pacific oysters, *Crassostrea gigas*: ripe yearling diploid oysters were in negative energy balance while triploids of the same age remained in a state of positive energy balance. Second, in shellfish such as oysters, the gonad ramifies throughout the somatic tissue often rendering ripe animals unmarketable. Depleted glycogen stores in the body also affect flavour. Triploid oysters overcome this problem (Allen and Downing 1991, submitted) and have enabled year-round production of *C. gigas* (Allen 1988a). Third, the potential to produce sterile triploids of non-native species enables their use as aquaculture organisms in areas which might be sensitive to the accidental introduction of competitor species.

As will be discussed in more detail later, the genetic consequences of the induction of triploidy depends, to some extent, on which of the two maturation divisions is

targeted. Theoretically, triploids induced by "chromosome-doubling" at meiosis I are generally likely to be more heterozygous across the whole genome than meiosis II induced triploids, and these would be more heterozygous than ordinary diploids. In principle, such high overall genomic heterozygosity would be expected to produce the well documented phenomenon of "hybrid vigour" or "heterosis" which may be manifested as faster growth, higher viability or generally increased fitness.

Apart from the purely commercial applications of triploid shellfish, such genetically manipulated organisms are also valuable in other ways. They may be used for studies on the physiology of growth (e.g. Tabarini 1984, Mason et al. 1988), the partitioning of energy between gonad and somatic tissue (e.g. Allen and Downing 1986, 1990), the production of maturation hormones or for genetic studies on recombination rates or "heterosis" (e.g. Allendorf and Leary 1984, Beaumont and Kelly 1989).

The value of triploid production, particularly in the case of the introduction of exotic species, is compromised because treatments are not 100% effective. This could be overcome by the use of tetraploid individuals as brood stock. A tetraploid female should produce exclusively diploid eggs which, when fertilized by normal haploid sperm, would be expected to give 100% triploid offspring. In spite of the apparent simplicity of this solution, successful techniques for the production of tetraploid shellfish have been elusive.

Diploid gynogenomes contain only chromosomes derived from the egg nucleus and, depending on the type of cell division suppressed, will exhibit either reduced heterozygosity or complete homozygosity. Gynogenesis is, therefore, a very efficient method for the rapid production of highly homozygous inbred lines. Normally, inbred lines are produced by matings between close relatives and crossing between such inbred lines can result in heterosis. This is a common and valuable feature of commercial production of agricultural animals and plants.

#### METHODOLOGY

The induction of triploids, tetraploids or diploid gynogens depends upon "chromosome-doubling" during either meiosis I, meiosis II or first cleavage, using physical or chemical shock treatment. In most molluscan shellfish any one of these three divisions can be targeted because the eggs are usually spawned just before, or at, the metaphase stage of meiosis I (Raven 1966). Furthermore, development is halted at this stage until eggs are activated by spermatozoa, enabling relatively precise control of the start of the maturation divisions. By contrast, it is not easy to target the first meiosis in most fish species because this division takes place in the ovary before egg release. Crustacean shellfish are even less amenable to ploidy manipulation, because most commercially important species do not release their eggs freely into the sea, but brood their eggs and

embryos. It is important to note that brooding oyster species (e.g. *Ostrea edulis*) are similarly less amenable to ploidy manipulation.

#### Triploidy

The normal course of the egg maturation divisions in bivalves is shown diagrammatically in Figure 1 (a-d). If physical or chemical shock treatment is administered at meiosis I, the homologous pairs of chromosomes destined to form the first polar body are retained within the egg nucleus (Fig. 1e). Meiosis II then follows and, assuming an equational division, the polar body formed should contain two sets of chromosomes and the egg should also be diploid (Fig. 1f). When the haploid sperm pronucleus fuses with the diploid egg nucleus a triploid zygote is formed (Fig. 1g). If meiosis II is targeted, the first polar body is allowed to form in the normal way. However, when the chromatids separate in meiosis II the second polar body is suppressed and this second set of chromatids is retained to form a diploid egg nucleus (Fig. 1h). This "diploid egg" can then produce a triploid zygote on the addition of the sperm nucleus (Fig. 1i).

When meiosis I proceeds normally, one chromosome of each homologous pair will be included in the first polar body, leaving a haploid egg. No allelic variation can exist in the haploid state and, in the normal course of events, potential variation is re-introduced by the addition of the male pronucleus during syngamy (Fig. 1c, d).

The potential allelic variation at a locus is increased in triploids. If three alleles (A, B and C) are present at a locus in a population, then triploids may be homozygous for one allele, heterozygous for two or heterozygous for all three. In theory, the possession of three, rather than two, alleles at a locus might be expected to have some effect on the fitness of an organism, but this is by no means certain and has yet to be demonstrated. Nevertheless, triploids should exhibit a general increase in two allele heterozygosity compared to diploids. Actual increases in heterozygosity at a locus depend not only on which meiotic division is suppressed, but also on the frequency of crossing-over (recombination) between the centromere and the locus (Table 1). Meiosis I triploids retain maternal heterozygosity at a locus when there is no recombination. However, with recombination, zygotic heterozygosity is reduced. On the other hand, meiosis II treatment, without recombination, produces a homozygous diploid egg: heterozygosity results when recombination occurs.

Without the effects of recombination, higher overall heterozygosity is expected from meiosis I triploids compared to meiosis II triploids and diploids. However, it is known that recombination frequencies are often high for many loci (Thompson and Scott 1984) and high levels of recombination will clearly erode the differences in overall heterozygosity between meiosis I and meiosis II triploids (Table 1). Furthermore, our example is based on a single female and a

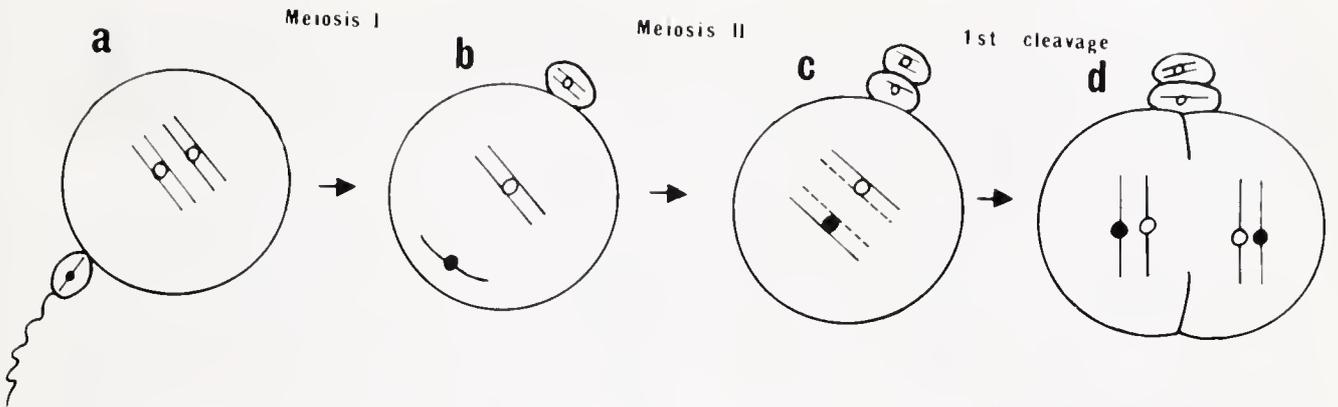


Figure 1. (a-d)—Normal development in the bivalve mollusc egg. For simplicity only one pair of chromosomes is shown. (a) egg at release at metaphase of meiosis I, activation by sperm; (b) meiosis I complete, first polar body extruded, sperm nucleus has entered egg; (c) meiosis II completed, second polar body extruded, male and female pronuclei unite; (d) first cleavage perpendicular to point of polar body extrusion.

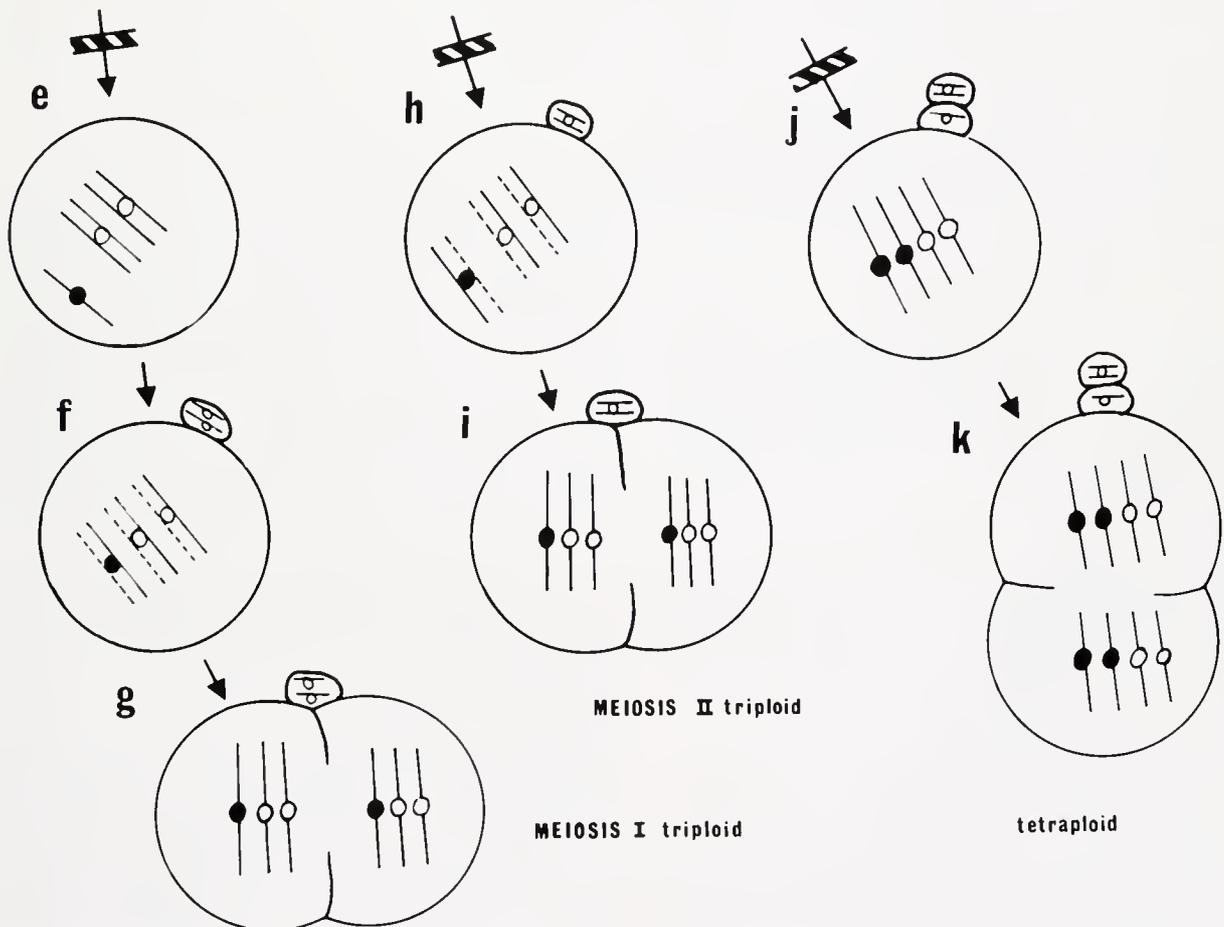


Figure 1. (e-k)—Ploidy manipulation. (e) shock administered during meiosis I, both chromosomes of the pair retained in the egg, first polar body not extruded; (f) normal meiosis II allowed, second polar body extruded, 2N female pronucleus and N male pronucleus unite; (g) triploid first cleavage. (h) shock administered during meiosis II, second polar body not extruded, 2N female pronucleus and N male pronucleus unite; (i) triploid first cleavage. (j) shock administered during first cleavage; (k) tetraploid chromosome complement in second cleavage (at right angles to first cleavage).

TABLE 1.

Potential genotypes of diploid eggs and triploid zygotes produced by suppression of meiosis I or II, with or without recombination. In this example, the female is heterozygous for two alleles, A and B, at a locus and the male is homozygous for B. Normal fertilization would be expected to produce equal numbers of AB and BB genotypes.

		Diploid Egg	Zygote
Meiosis I	without recombination	AB	ABB
	with recombination	AA AB BB 1: 2: 1	AAB ABB BBB 1: 2: 1
Meiosis II	without recombination	AA BB 1 : 1	AAB BBB 1 : 1
	with recombination	AB	ABB

single male: when many progenitors are used the levels of heterozygosity will depend on both recombination frequencies and allele frequencies (Allendorf and Leary 1984). Finally, it is assumed that a true equational meiosis II division follows the suppression of meiosis I. This has yet to be demonstrated. Indeed, evidence from Guo et al. (1989b) and also from our own observations (unpublished data) suggest that aneuploidy may result from meiosis I treatment and that cytoskeletal and chromosomal development of many eggs treated at either meiosis I or II is abnormal. Further research is needed to clarify the type of division which follows suppression of meiosis I.

### Tetraploidy

#### (i) Suppression of First Cleavage

Theoretically, if treatment is administered during first cleavage, the chromosomes will divide to form two sets but the cell will not cleave, resulting in one cell with double the diploid number of chromosomes (Fig. 1j, k). Subsequent mitotic divisions then should produce tetraploid cells.

#### (ii) Blastomere Fusion

This novel method has produced a very low percentage of tetraploidy in *Crassostrea gigas* (Guo et al. 1988). Embryos at the two-cell stage are treated with 50% polyethylene glycol to induce fusion of the blastomeres, rinsed in seawater and then cultured as normal.

#### (iii) Early Shock Treatment

In some triploidy trials (e.g., Stephens & Downing 1988, Cooper and Guo 1989, Guo et al. 1989b; Diter and Dufy 1990) a significant number of tetraploids have been induced by shock treatment administered immediately after sperm activation. It is not clear how this can produce tetraploids, but Diter and Dufy (1990), working with the Manila clam, *Tapes philippinarum*, suggest that treatment with CB at the time of fertilization may delay sperm penetration into the egg cytoplasm without inhibiting egg activation. When CB is removed, sperm entry would be expected to proceed

normally, but formation of the first cleavage spindle would be delayed and unavailable when the chromosomes were ready for first mitosis, resulting in an inhibition of the first mitotic cleavage, thus leading to tetraploidy.

### Diploid Gynogenesis

If egg maturation is initiated by sperm which has been gamma- or ultra violet light irradiated to destroy the sperm DNA, the egg will remain haploid (Fig. 2a-d), as will any embryos which develop from such eggs. However, shock treatment can be targeted at meiosis I, meiosis II or first cleavage to restore diploidy (Fig. 2e-g). Heterozygosity of diploid gynogens is reduced compared to maternal heterozygosity and is dependent on division treated and recombination (see diploid egg column, Table 1). In theory, completely homozygous diploid gynogens are only produced when first cleavage is suppressed.

### Agents of Induction

#### (i) Chemical Shock

Cytochalasin B (CB), one of a class of fungal metabolites, is thought to inhibit micro-filament formation in cells (Copeland 1974) and is the most commonly used chemical shock treatment to induce "chromosome-doubling" in bivalves. In the maturation divisions, polar body formation can be divided into two stages, the first being the extrusion of a portion of cytoplasm containing maternally derived chromosomes. According to Longo (1972), this stage is unaffected by CB, but CB prevents the development of a cleavage furrow at the base of the protrusion. This results in the retention of the set of chromosomes that would have formed such polar bodies (Longo 1972). In cells undergoing mitosis, prophase and metaphase progress normally, but, at the onset of telophase, cells exposed to CB show neither the gross morphological presence of a cytoplasmic cleavage furrow, nor the filaments of the contractile ring apparently requisite for effective cytokinesis (Copeland 1974).

Cytochalasin B is hydrophobic and is, therefore, dissolved in DMSO (dimethyl sulphoxide) as a carrier solution (Allen 1987b), before being made up to the desired concentration (0.1–1.0 mgCB/l) with filtered sea water. Fertilized eggs are held in this solution at the appropriate time. At the end of treatment, which usually lasts 15–20 minutes, eggs are transferred to a solution of DMSO in filtered sea water (0.01–0.1%) for 15–20 minutes to remove the remaining CB. Thereafter, eggs are returned to filtered sea water and reared in the normal way. For a practical description of the methodology used to produce meiosis II triploid oysters using CB, the reader is referred to Allen et al.'s excellent Hatchery Manual (1989).

#### (ii) Thermal Shock

Both heat and cold shocks can be used to induce "chromosome-doubling" at meiosis I, meiosis II or first

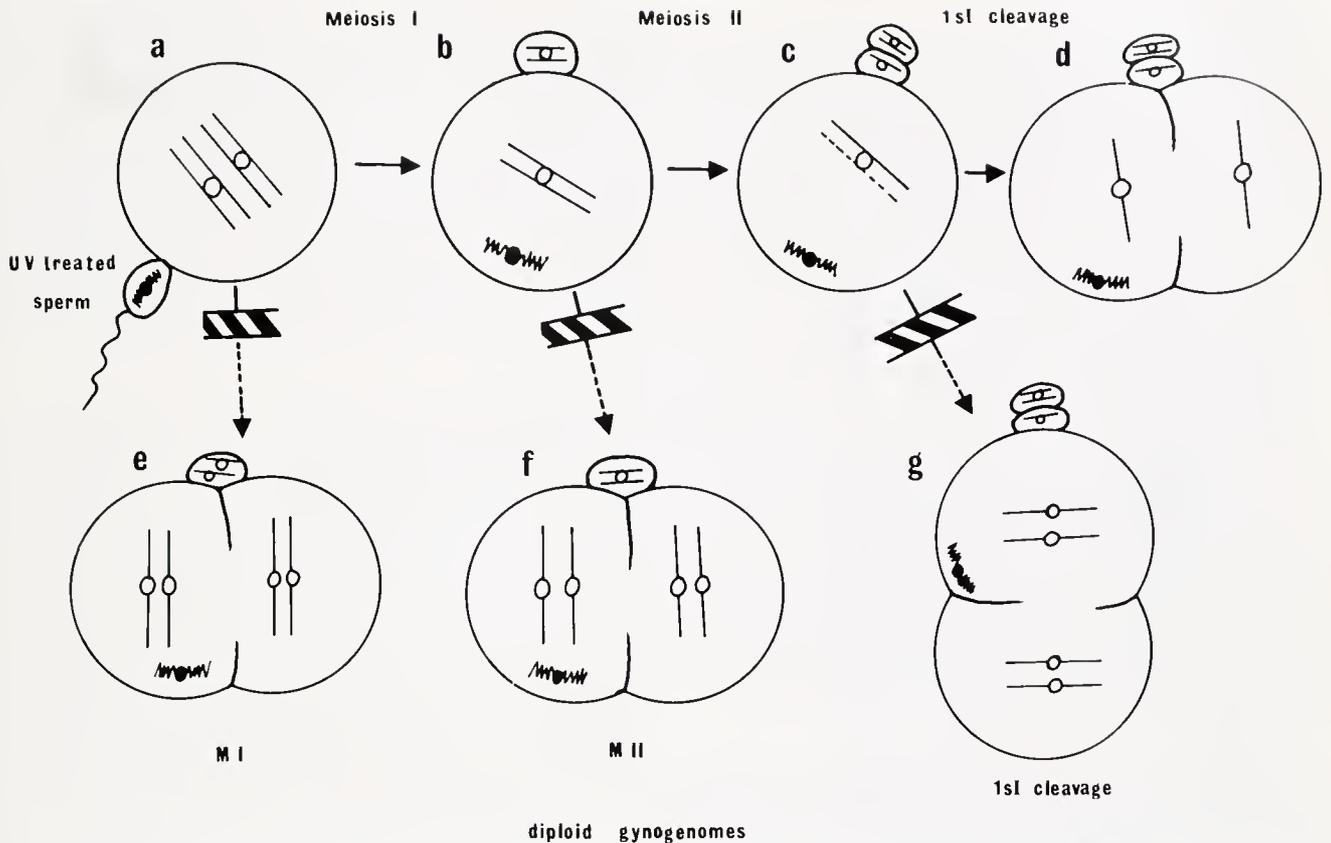


Figure 2. Gynogenesis in bivalve molluscs. For simplicity, only one pair of chromosomes is shown. (a–d) maturation divisions and first cleavage of egg after activation by UV treated sperm. Diploid gynogenomes produced by shock administered at meiosis I (e), meiosis II (f) or first cleavage (g).

cleavage. Effective heat shock temperatures range from 25–38°C and cold shocks from 0–5°C. The actual differential between normal incubation temperature and the thermal shock temperature is likely to be of more importance than the absolute temperature of the treatment (Allen 1987). A combined heat and chemical (caffeine) shock technique has recently been developed by Yamamoto et al. (1990) and Scarpa et al. (1991, submitted).

### (iii) Pressure Shock

Pressure shocks have been successfully used to produce triploid *Crassostrea gigas* (Chaiton and Allen 1985, Allen et al. 1986a) and triploid abalones, *Haliotis discus hannai*, (Arai et al. 1986). Pressure is applied by placing activated eggs into a suitable container which is then subjected to 6000–8000 psi for 10 minutes (Chaiton and Allen 1985). These extremely high pressures interfere with normal meiotic or mitotic cell divisions. When pressure is released, development resumes and eggs or zygotes can be removed and reared under normal conditions.

### VERIFICATION OF PLOIDY

Early assessment of treatment success ensures that time and space will not be wasted rearing batches of larvae with low yields of polyploids. The main methods of ploidy as-

essment used for molluscs are chromosome counts, polar body counts, flow cytometry, microfluorometry, nuclear sizing by microscope or Coulter counter, and electrophoresis. Practical details of most of these techniques are given in Allen et al. (1989).

### Chromosome Counts

The most direct method of verification is chromosome counts, which can also be used to compare between other verification techniques. Direct counts of chromosomes can be made at various stages of development allowing eggs and embryos to be screened for ploidy. Tissue from spat, juveniles and adults can also be used. Standard techniques involve fixing eggs, taken at appropriate times, directly in Carnoy's fixative (3 parts alcohol:1 part glacial acetic acid). Embryos are first treated with Colchicine in filtered sea water before transfer to 25% dilute sea water and final fixation. Colchicine arrests mitosis at the metaphase stage and increases the number of metaphase plates available for chromosome counts. This method of ploidy assessment is time consuming and it is not uncommon to find that the majority of the embryos examined on a slide do not provide countable chromosome spreads for one reason or another. Nevertheless, chromosome counts do provide a direct method of ploidy verification.

### Polar Body Counts

Counting the number of polar bodies associated with zygotes and very early embryos has been suggested as a rapid and simple method for assessing the success of triploidy in bivalves (Beaumont 1986). Because triploidy induction involves the suppression of either the first or the second polar body, triploid zygotes should have only one polar body, not two. This method appears to have some value with the scallop, *Pecten maximus*, (Beaumont 1986) and the pearl oyster, *Pinctada radiata* (S. A. A. Khamdan, Bahrain Environmental Protection, Bahrain, pers. comm.), but seems unsuitable for verification of ploidy in Manila clam, *Tapes semidecussatus*, or the mussel, *Mytilus edulis*, due to difficulties in recognising and recording polar bodies associated with early embryos (Beaumont and Contaris 1988, Beaumont and Kelly 1989). Stephens and Downing (1988) have used DAPI (see later) to investigate the ploidy of the polar bodies, in treated *Crassostrea gigas* eggs and early cleaving embryos. Since the first polar body is diploid and the second haploid, this method can determine which maturation division has been affected if only one polar body is visible at first cleavage.

### Flow Cytometry

Flow cytometry involves staining the nuclei of cells with a DNA-RNA specific fluorescent dye, such as propidium iodide or DAPI. Stained cells, in liquid suspension, are then laser excited and pass through the sensing zone of a cytofluorographic analyser, where the intensity of fluorescence is recorded (Allen 1983). Both haemolymph and fresh mantle tissue, as well as siphon, foot or mantle tissue which has been frozen for some time, have been used to provide cells for assay (Allen 1983). Samples are always run together with a standard, usually chicken erythrocytes, so as to determine relative DNA content. The modal values of fluorescence pulse-height histograms are recorded. Ideally, triploid modal values should be 1.5 times greater than diploid values. Flow cytometry has proved to be a fast and accurate procedure, which does not always necessitate killing the adult animal (Allen 1983) and has been used successfully with larvae as small as 250  $\mu\text{m}$  (Chaiton and Allen 1985) and, more recently, with trochophore larvae (Downing 1989b). A drawback of this technique is the very high price of flow cytometers, but it is often possible to have samples analyzed at institutes which do have such facilities.

### Microfluorometry with DAPI Staining

DAPI, 4'-6-diamidino-2-phenylindole, is an aromatic fluorochrome which binds preferentially to the adenine-thymine base pairs of DNA. Nuclei stained with DAPI are excited with ultra-violet light (365 nm) and the fluorescence intensity measured with a photometer. A histogram of fluorescence intensity (representing relative DNA content) produced from a sample containing, for example, ha-

plaid, diploid and triploid cells would show three distinct peaks (Komaru et al. 1988).

### Nuclear Sizing

The nuclei of triploid cells are 1.5 times the volume of diploid nuclei and, therefore, have a greater diameter. This difference in diameter can be measured, directly on a microscope, or using a Coulter counter. This technique has been successfully used in determining ploidy of fish (Johnson et al. 1984) and is now being developed for use with shellfish (A. R. Child; MAFF, pers. comm.).

### Electrophoresis

Electrophoresis is not widely used to verify ploidy, but Allen et al. (1982) have compared the effectiveness of electrophoresis and chromosome counting as verification tools in *Mya arenaria*. Large sample sizes can be processed rapidly and diploid genotypes are distinguished from triploid genotypes either on the basis of relative staining intensities of electromorphs, or by the presence of three electromorphs. However, only highly heterozygous loci give useful data. This technique is particularly useful when polyploidy has been induced with the aim of increasing or decreasing the incidence of heterozygosity, since it provides data on ploidy and heterozygosity at the same time.

## RESULTS

Ploidy manipulations have been carried out successfully in a number of bivalve species and one gastropod species, using chemical, thermal and pressure induction. Table 2 sets out, species by species, the methods which have been used for induction and ploidy assessment, together with the results obtained and other aspects covered by various workers. It is important to note that where the percentage of triploidy induction is less than 100%, offspring reared through the larval stage, and beyond, will consist of a mixture of diploids and triploids. Furthermore, the proportions of the two ploidy types may change during development due to differential mortality.

### *Crassostrea gigas* (Pacific Oyster)

#### Triploidy

##### (i) Chemical shock

Chemical induction using cytochalasin B (CB) has proved to be effective in the production of triploid Pacific oysters (Table 3). A dosage of 1 mgCB, dissolved in 1 ml DMSO, per litre of sea water has resulted in best triploid production ranging from 50–100% (mean 83%).

According to Downing and Allen (1987), the major factors influencing the effectiveness of CB treatment are the dosage, the duration of treatment, the temperature at which treatment is conducted and the time, after egg activation by sperm, at which treatment is applied. Sperm quality may also be important for successful triploidy in-

TABLE 2.

Ploidy manipulation in molluscs. 2N = diploid, 3N = triploid, 4N = tetraploid, MI = meiosis I, MII = meiosis II. Induction methods: CB = cytochalasin B, Ca = caffeine, C = cold, H = heat, P = pressure, C.F. = cell fusion. Verification methods: c.c. = chromosome counts, p.h.c. = polar body counts, f.c. = flow cytometry, m.f. = microfluorometry, elec. = electrophoresis.

Species	2N	Best %		Induction Method	Verification Method	Special Aspects Covered	Authors
		3N	4N				
<b>OYSTERS</b>							
<i>Crassostrea gigas</i> (Pacific oyster)	20	57	—	P	f.c.; c.c.	verification of larvae at only 250 µm long	Chaiton & Allen (1985)
		96	—	CB	f.c.	growth; gonadogenesis	Allen & Downing (1986)
		100	—	CB	f.c.		Allen et al. (1986a)
		60	—	P	f.c.		
		60	—	H	c.c.		Quillet & Panelay (1986)
		—	—	CB	f.c.	gametogenesis	Allen (1987a)
		90	—	CB	f.c.	optimal temperature for treatments	Downing & Allen (1987)
		—	—	—	f.c.	gonadogenesis; sex ratios	Allen (1988b)
		69	—	—	—	weight loss & glycogen utilization	Davis (1988a)
		—	—	—	—	energy budget analyses; gametogenesis	Davis (1988b)
		50	—	CB	f.c.	hybrids with <i>C. rivularis</i> crosses; sperm quality/induction success	Downing (1988a)
		—	—	—	—	hybrids with <i>C. rivularis</i> gametogenesis	Downing (1988b)
		—	4	C.F.	m.f.	fusion of oocytes by polyethylene glycol	Guo et al. (1988)
		75	91	CB	f.c.; m.f.	early shock produces tetraploids	Stephens & Downing (1988)
		67	—	CB	c.c.	growth rate of MI/MII	Yamamoto et al. (1988)
		83	—	H	c.c.	triploids compared to controls	
		67	—	C	c.c.		
		—	—	CB	f.c.; c.c.	timing of shock leads to triploids, tetraploids or pentaploids	Cooper & Guo (1989)
		—	—	—	—	gonadogenesis; growth; energetics	Davis (1989)
		100	—	CB	—	hybrids with <i>C. virginica</i>	Downing (1989a)
—	—	C.F.	—	cell fusion using polyethylene glycol; polyploid mosaics	Guo et al. (1989a)		
—	—	CB	f.c.; c.c.	pentaploids; aneuploids; chromosome segregation	Guo et al. (1989b)		
—	—	CB	—	gametogenesis; sexual expression; hermaphroditism	Allen & Downing (1990)		
—	—	H & Ca	—	synergistic effect of caffeine on heat shock	Yamamoto et al. (1990)		
<i>Crassostrea virginica</i> (American oyster)	20	—	—	—	—	gynogenesis	Stiles (1978)
		50	—	CB	c.c.	triploid and tetraploid embryos	Stanley et al. (1981)
		—	—	—	f.c.	detailed methodology for flow cytometric ploidy analysis	Allen (1983)
		—	—	—	—	gynogenesis and androgenesis	Stiles et al. (1983)
		72	—	CB	f.c.	growth rate/heterozygosity	Stanley et al. (1984)
		—	—	CB	f.c.	gametogenesis; sex ratio	Allen (1987a)
		—	—	—	—	gametogenesis	Lee (1988)
100	—	CB	—		Shatkin & Allen (1989)		

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TABLE 2.  
continued

Species	2N	Best %		Induction Method	Verification Method	Special Aspects Covered	Authors
		3N	4N				
<i>Pinctada fucata</i>	28	65	—	CB	c.c.; m.f.		Uchinura et al. (1989) Wada et al. (1989)
<i>martensii</i>		—	—	C	c.c.; m.f.		
(Japanese pearlyster)		100	—	CB	m.f.		
		52	—	C	m.f.		
		—	—	H	m.f.		
SCALLOPS							
<i>Argopecten irradians</i>	32	94	—	CB	f.c.	growth; sterility	Tabarini (1984)
(Bay scallop)							
<i>Chlamys nobilis</i>	32	88	—	CB	c.c.; f.c.; m.f.	karyotype of diploid and triploid scallops; verification methods	Komaru et al. (1988)
		71	—	CB	m.f.	gametogenesis & growth	Komaru & Wada (1989)
<i>Chlamys varia</i>	38	78	—	CB	c.c.	growth; karyotype	Baron et al. (1989)
(Black scallop)							
<i>Pecten maximus</i>	38	30	—	CB	c.c.; p.b.c.	genetic variability; hatchery rearing; self-fertilization	Beaumont (1986)
(Great scallop)							
MUSSELS							
<i>Mytilus edulis</i>	28	85	—	C	c.c.		Yamamoto & Sugawara (1988)
(Mussel)		97	—	H	c.c.		
		25	—	H	c.c.	growth rate	Beaumont & Kelly (1989)
		67	—	CB	c.c.		Yamamoto et al. (1990)
		—	—	H & Ca		synergistic effect of caffeine on heat shock	
		—	—	H & CaCl <sub>2</sub>		and of CaCl <sub>2</sub> on heat shock	Fairbrother & Beaumont (unpubl.)
		54	—	CB	c.c.	mass mating; growth rate	
		—	—	—	c.c.	gynogenesis	_____ (unpubl.)
CLAMS							
<i>Mercenaria mercenaria</i>	—	—	—	CB	f.c.		Buzzi & Mannzi (1988)
(Hard-shelled clam)						growth rate	Hidu et al. (1988)
<i>Mulinia lateralis</i>	38	—	—	CB	c.c.	growth rate	Rupright (1983)
(Dwarf surf clam)						54% 2N gynogenomes to 2-cell stage; parthenogenesis; triploids; tetraploids	Scarpa (1985)
		—	—	H & Ca; CB	m.f.; f.c.	parthenogenetic diploids	Scarpa et al. (1991) submitted
<i>Mya arenaria</i>	34	100	—	CB	c.c.; elec.	use of electrophoresis to check ploidy	Allen et al. (1982)
(Soft-shelled clam)						detailed methodology for flow cytometric analysis of ploidy	Allen (1983)
		89	—	CB	elec.; f.c.	gametogenesis; sex ratio	Allen et al. (1986b)
		—	—	CB	f.c.	gametogenesis; sex ratio	Allen (1987a)
		44	—	CB	f.c.	heterozygosity; energy budget	Mason et al. (1988)
<i>Tapes philippinarum</i>	38	75	—	CB	c.c.	growth rate	Dufey & Diter (1990)
(= <i>Ruditapes philippinarum</i> / = <i>Tapes semidecussatus</i> )		—	64	CB	c.c.	early shock produces tetraploids	Diter & Dufey (1990)
(Manila clam)		68	—	CB	c.c.		Beaumont & Contaris (1988)
		55	—	H	c.c.		Gosling & Nolan (1989)
		50	—	CB	c.c.		

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TABLE 2.  
continued

Species	2N	Best %		Induction Method	Verification Method	Special Aspects Covered	Authors
		3N	4N				
GASTROPODS							
<i>Haliotis discus hannai</i> (Pacific abalone)	36	—	—	—	c.c.	gynogenetic activation of eggs	Arai et al. (1984)
		80	—	C	c.c.;	karyotype for diploids and triploids given	Arai et al. (1986)
		80	—	H	c.c.;		
		60	—	P	c.c.;		
		72	—	C	c.c.; elec.	temperature tolerant and thermo resistant allele frequencies studied	Fujino et al. (1987)
		87	—	C	c.c.; elec.	effect of maternal genotype at PGM locus on differential contribution of oocytes to triploid offspring	Fujino et al. (1988a)
		86	—	C	c.c.; elec.	effect of maternal genotype at multiple loci on differential contribution of oocytes to triploid offspring	Fujino et al. (1988b)
—	—	—	—	—	gynogenetic diploids	Fujino et al. (1990)	

duction because ripe sperm ensures rapid activation and is more likely to yield synchronously developing eggs (Downing 1988a).

Downing and Allen (1987) investigated the effect of temperature on CB treatment and determined the optimal treatment for producing a high proportion of triploids and acceptable larval survival. Best results were achieved by treatment beginning at 30 min. and ending at 45 min. post-activation at 25°C. Probably only meiosis II was affected because meiosis II occurs between 40–55 min. post-activation at 18°C and development would be faster at 25°C (Stephens and Downing 1988). *C. gigas* triploids have been produced by targeting both meiosis I and II (Downing and Allen 1987, Stephens and Downing 1988, Cooper and Guo

1989, Guo et al. 1989b). Diploid and triploid hybrids have been produced from crosses between *C. gigas* × *C. rivularis* (Downing 1988b) and *C. gigas* × *C. virginica* (Downing 1989a).

#### (ii) Thermal shock

In contrast to its use in fish ploidy manipulation (e.g. Thorgaard 1983), thermal shock has not been extensively used in bivalves. However, triploid *C. gigas* have been induced by heat shock over a range of temperatures (30–40°C), duration times (3–20 min) and starting times (10–60 min post-activation) by Quillet and Panelay (1986) and Yamamoto et al. (1988). Longer shocks (20 min duration) were more effective than shorter ones (10 min) and

TABLE 3.

Treatment regimes for chemically induced triploidy in *Crassostrea gigas*. CB = cytochalasin B, min.p.a. = minutes post-activation, MI = meiosis I, MII = meiosis II, n.g. = not given.

Dosage (mgCB/l)	Start Time (min p.a.)	Duration (min)	Temp. (°C)	Best Results (%)	Treatment	Authors
1.0	30	15	20	96.3	—	Allen & Downing (1986)
1.0	0–45	15	18–28	100	30–45 mns p.a. at 25°C	Allen et al. (1986a)
1.0	0–120	15	18–25	90	30–45 mns p.a. at 25°C	Downing & Allen (1987)
n.g.	30	15	—	50	—	Downing (1988b)
n.g.	15–55	15	18	75	40–55 mns pa.	Stephens & Downing (1988)
0.1–1.0	10–30	20	19	67.2	0.5 mgCB/l at 10 mns p.a.	Yamamoto et al. (1988)
n.g.	MI MII	—	25	—	—	Cooper & Guo (1989)
n.g.	20	15	25	100	—	Downing (1989a)
n.g.	MI MII	—	25	—	—	Guo et al. (1989b)

there was little effect on early survival of putative triploids, except at higher treatment temperatures (38°C) (Quillet and Panelay 1986). Yamamoto et al. (1988) reported 66.7% triploidy induction by applying cold shock (0°C) for 10 min duration starting at 15 min post-activation.

If thermal shock could produce triploid yields comparable to those resulting from use of cytochalasin B, this would provide an easier and safer method of induction. More research is needed to confirm the high yields (83.3%) obtained by Yamamoto et al. (1988). Allen (1987b) points out that, since thermal treatment arrests all development, only those eggs which are at a vulnerable stage of cell division at the time of shock will be affected by the treatment. By contrast, CB does not appear to interrupt development in the same way and eggs will continue to develop, during treatment, until they reach a vulnerable stage of cell division. It therefore seems unlikely that thermal induction alone will be able to reliably and repeatedly produce high, commercially viable, percentages of triploid oysters. Recently, Yamamoto et al. (1990) have reported increased yields of triploid *C. gigas* using a combination of heat shock (32°C) and caffeine (10 mM), compared to heat shock alone.

### (iii) Pressure Shock

Hydrostatic pressure shocks have similar limitations to heat treatment in that they arrest all development. Nevertheless, Chaiton and Allen (1985) and Allen et al. (1986a) have produced triploid *C. gigas* (10–60%) using 6000–8000 psi pressure shocks of 10 min duration applied 10 min post-activation. Best yields of 60% were achieved by applying a shock of 7200 psi at 29°C (Allen et al. 1986a). Unlike chemical and heat treatment methods, a specially manufactured, non-toxic, pressure vessel, capable of safely withstanding the very high pressures used, is a prerequisite for this type of treatment.

### Tetraploidy

Although targeting of meiosis I and II with CB is designed to produce triploids, such treatment has also been found to induce tetraploidy under certain circumstances (Stephens and Downing 1988, Cooper and Guo 1989, Guo et al. 1989b). Stephens and Downing (1988) treated *C. gigas* eggs with cytochalasin B from 15–30 min post-activation, at the low temperature of 18°C and produced an estimated 9% diploids and 91% tetraploids by chromosome counts at 24 hours. Similar treatment, targeting meiosis II resulted in counts of 8% diploids, 75% triploids and 17% tetraploids at 24 hours. However, tetraploids produced in this way suffer high mortality during early development and no tetraploid spat resulted from these treatments. Similarly Cooper and Guo (1989b) reported 100% mortality of tetraploids induced in this way within 72 hours post-activation. It is not clear how or why tetraploids are produced

under these circumstances and further research is required to explain these results.

Guo et al. (1988, 1989a) reported a novel method of production of up to 4% tetraploids by using polyethylene glycol at the 2-cell stage to bring about oocyte fusion. Although the percentage of tetraploids produced is very low, it will be interesting to see if the technique can be further developed to increase its success.

### Pentaploidy

Cooper and Guo (1989) have produced *C. gigas* pentaploids by sequential cytochalasin B inhibition of both meiosis I and meiosis II. In this way, two "chromosome-doubling" events are induced in the egg, to give a tetraploid female pronucleus which becomes pentaploid on the addition of the male pronucleus. Cooper and Guo (1989) found that pentaploid zygotes developed into abnormal trochophores, failed to develop to D-stage larvae and were all dead within 72 hours from egg activation.

### Gynogenesis

In a recent review, Allen (1987b) refers to the successful activation of 30% to 88% *C. gigas* eggs by ultra-violet light irradiated sperm. However, as yet, there are no reports of successful production of oyster diploid gynogens using such irradiated sperm.

### Survival

Different treatment methods have different effects on survival of early larval stages. The use of CB always leads to reduced survival up to D-larval stage compared to controls. Furthermore, Downing and Allen (1987) found that the effect on mortality was greater when CB was administered during critical stages of development, such as, activation, polar body formation and cleavage, compared to non-critical times. However, Quillet and Panelay (1986) found no such depressive effects on 24 hour larvae treated by heat shock, except at the highest treatment temperature (38°C). Reduced survival due to CB can also be exacerbated by other factors such as, for example, quality of strip-spawned eggs (Downing and Allen 1987) or limitations of the larval rearing system (Quillet and Panelay 1986). As noted earlier, tetraploids produced by targeting meiosis I or II and pentaploids appear to have an ephemeral existence (Stephens and Downing 1988, Cooper and Guo 1989).

Once the D-larval stage has been reached, triploids exhibit similar mortality rates to diploids (Downing and Allen 1987). However, ploidy itself can have effects on survival at later stages. When reproductively active diploid and triploid Pacific oysters were starved for 130 days, diploid survivorship was greater than that of triploids. Weight loss and reduction in glycogen content in triploids exceeded that of diploids, despite triploids having higher initial glycogen levels (Davis 1988a).

## Physiology

### (i) Gonadogenesis and Sex Ratio

Production of sterile oysters which maintain a positive energy balance during the reproductive season and can be marketed all year round is the main commercial aim of triploidy induction (Allen 1988a). In fact, the induction of triploidy in bivalves does not necessarily bring about complete sterility, but rather a reduction in gonadogenesis (Allen and Downing 1986, Allen 1987a, Allen 1988a, 1988b). The extent of gametogenesis in triploid *C. gigas* is sexually dependent, as is generally the case in triploid fish species (Thorgaard 1983). Male gonadal development is more extensive than female, the males often producing substantial populations of spermatocytes but not spermatozoa. Females produce few oocytes, most females producing undeveloped follicles containing rudimentary gonial cell populations (Allen and Downing 1986, Allen 1987a, Allen 1988b, Allen and Downing 1990). Cytofluorometric analysis has shown that some meiosis does occur in triploid males, resulting in aneuploid gametes (Allen 1987a, 1987b, Allen 1988b). Despite retarded gonad development, triploid oysters have been observed to spawn both male and female gametes (Allen 1988b, Allen and Downing 1990).

Allen and Downing (1990) reported the sex ratio of yearling diploid *C. gigas* to be 1.6 males to 1 female. Excluding hermaphrodites, sex ratios in cohorts of diploids and triploids are similar (Allen 1987a, Allen 1988b). Allen and Downing (1990) discuss the unexpected increased incidence of hermaphrodites among triploid oysters (29%) compared to diploids (1%), and the change in their frequency in the population as maturity reaches a peak.

### (ii) Glycogen Levels

Gametogenesis and glycogen utilization are intimately associated in bivalves (Gabbott 1975). The normal pattern of glycogen level in diploids is one of a steady decline throughout the gametogenic period, followed by an increase after spawning. Triploids do not show this seasonal drop in levels, retaining high glycogen throughout the season (Allen and Downing 1986, Allen 1987a, Downing 1988b). Downing (1988b), comparing glycogen levels among diploid and triploid *C. gigas* and *C. rivularis* and their hybrids, found that, in general, triploids had higher glycogen levels than diploids during gametogenesis.

### (iii) Growth Rate

Larval growth rate is generally similar for diploids and triploids (Downing and Allen 1987). Allen (1988a) reports that triploid oysters do not appear to grow faster during their first year, but do so in the second year and suggests two reasons for this. First, reproductive effort generally increases in the second year, so that any differences in growth rate between fertile diploids and sterile triploids is

accentuated at that time. Secondly, oysters change sex during their lifetime. They are usually male first, and female later. Since triploid females produce far fewer gametes than triploid males, a larger proportion of the population, in the second year, will have a lower reproductive output.

Differences in growth rate between diploid and triploid oysters can also be affected by environmental factors. Over a two year period Davis (1989) compared growth rate of diploid and triploid oysters at two sites of similar productivity but whose temperature maxima were different (20 and 16°C). Triploids grew faster than diploids at both sites, the difference being greater at the site with the higher maximum temperature.

Yamamoto et al. (1988) have reported that the mean shell length of 25-day-old triploid larvae induced by heat shock at meiosis I was significantly greater than that of meiosis II triploids or controls. Feeding and digestion rates amongst these same animals were not significantly different. Increased growth rate of meiosis I against meiosis II larval triploids might be due to the theoretical increased overall heterozygosity produced by targeting meiosis I.

## *Crassostrea virginica* (American Oyster)

### Triploidy

Stanley et al. (1981) were the first to attempt triploidy production in bivalves, using *C. virginica*. A variety of CB treatment regimes have now been used with varying degrees of success. Dosages ranging from 0.1–5.0 mgCB/l, lasting 10–20 min, starting at 1–50 min post-activation at temperatures ranging from 25 to 29°C, have all been attempted (Stanley et al. 1981, Stanley et al. 1984, Shatkin and Allen 1989). Stanley et al. (1984) timed treatments of 0.5 mgCB/l to coincide with meiosis I and II (0–15 and 15–30 min post-activation at 28–29°C) and obtained 61% meiosis I and 72% meiosis II triploids. Very high percentages of triploids were obtained using a dose of 0.5 mgCB/l for 15 minutes starting at 25 min post-activation at a temperature of 25°C (96%) (Shatkin and Allen 1989). These authors also treated a commercial spawn using this procedure and obtained 100% triploidy.

### Gynogenesis

A cytological appraisal of the prospects for gynogenesis and androgenesis, using ultra-violet light irradiation of sperm and oocytes, in *C. virginica* has been undertaken by Stiles et al. (1983), following earlier work using X-irradiation to inactivate sperm DNA (Stiles 1978). It is important to establish the appropriate dosages of UV or X-irradiation for sperm. Too much will prevent the sperm from being able to activate the eggs; too little will allow some integration of sperm chromosomes into the zygote nucleus. At higher levels of X-irradiation (15,000R to 225,000R),

sperm generally failed to activate eggs, more than 75% remaining at metaphase of meiosis I (Stiles 1978). However, sperm X-irradiated at 10,000R activated up to 66% of the eggs to undergo maturation divisions and these eggs proceeded as far as first cleavage, but no further, with severely, visibly damaged, sperm chromosomes contributing to the developing zygote (Stiles 1978).

Stiles et al. (1983) carried out UV-irradiation of sperm using an 8 watt UV lamp, wavelength 254 nanometers and this was found to be more effective than X-irradiation. Exposures of sperm to between 1.5 and 4 min UV irradiation allowed activation of more than 50% of the eggs, such eggs having a haploid female pronucleus and two polar bodies. These results demonstrate the potential for the production of diploid gynogens in *C. virginica* although initial trials did not include a "chromosome-doubling" step. Of interest also is the demonstration of androgenesis, whereby, UV irradiated eggs can be activated by normal sperm (Stiles et al. 1983).

#### Survival

As with *C. gigas*, CB treatment of *C. virginica* leads to decreased survival during early development compared to diploids, with fewer veliger larvae being produced (Stanley et al. 1981). Mortality was also strongly correlated with the timing of CB treatment. Early treatment, just after activation, gave reasonable survival, though less than controls, while treatment administered at 40 min post-activation produced high mortalities.

#### Physiology

##### (i) Gonadogenesis and Sex Ratio

The nature and degree of maturity reported for adult triploid *C. virginica* is similar to that observed in triploid *C. gigas*. Male triploids develop large populations of spermatocytes, some develop spermatids and a few produce spermatozoa. Female triploids show arrested oogonial differentiation with the development of small numbers of oocytes, few of which ripen (Allen 1987a, Lee 1988). Similarly, triploid *C. virginica* show an increased incidence of hermaphroditism compared to diploids, and, excluding hermaphrodites, there are, again, no significant differences in the sex ratios of diploid and triploid cohorts (Allen 1987a).

##### (ii) Growth rate

Stanley et al. (1981) reported that cohorts of triploid *C. virginica* set normally and grew at least as well as controls up to 8 months of age, at which time there was no significant difference between the size of diploids and triploids. However, they later showed (Stanley et al. 1984) that two-year-old meiosis I triploid oysters grown in the field were significantly larger than their meiosis II triploid and diploid counterparts. This was the earliest demonstration of possible heterosis resulting from increased heterozygosity of meiosis I triploids, and these authors were the first to point

out the different genetic consequences of targeting meiosis I and meiosis II. In order to investigate actual heterozygosity, they scored six enzyme loci using starch gel electrophoresis and reported highest heterozygosity in the meiosis I triploids. Furthermore, the percentage of loci that were heterozygous was 38% in meiosis I triploids but only 26% in meiosis II triploids and diploid controls. It is surprising that measured heterozygosity in meiosis II triploids was not greater than in diploids, since recombination would be expected to effect at least some of the loci scored.

#### *Pinctada fucata martensii* (Japanese Pearl Oyster)

##### Triploidy

Two recent studies by Uchimura et al. (1989) and Wada et al. (1989) have demonstrated that treatment with 0.5 and 0.1 mgCB/l from 20 to 50 min post-activation can produce an average of 90% triploid juvenile Japanese pearl oysters. These authors have also experimented with heat and cold shock triploidy induction. An upward differential of 11°C and a downward differential of 17.5°C have both proved capable of inducing "chromosome-doubling" during the maturation divisions, but neither of these thermal techniques were able to produce the high percentages of triploids obtained using Cytochalasin B.

Initial trials on the Arabian gulf pearl oyster (*Pinctada radiata*) using CB have also demonstrated the potential for this technique to produce triploid *P. radiata* larvae (S. A. A. Khamdan, Bahrain Environmental Protection, Bahrain, pers. comm.).

#### *Argopecten irradians* (Bay Scallop)

##### Triploidy

Triploidy was induced in *A. irradians* by Tabarini (1984) after treating newly activated eggs with CB. Treatment for 20 min duration, 10 min post-activation resulted in 66% triploidy using 0.05 mgCB/l and 94% triploidy using 0.1 mgCB/l.

##### Physiology

When yearling scallops were grown on in the field, mean adductor muscle weight and total body tissue wet weight were greater in triploids than in diploids (73% and 36% respectively). The majority of triploid scallops failed to ripen during the summer months, and the relationship between increased growth, stage of reproductive cycle and sterility in triploids is discussed by Tabarini (1984). It is probable that the large reported differential in growth between triploids and diploids was due to the lack of gonadic growth in triploids.

#### *Chlamys nobilis*

##### Triploidy

Komaru et al. (1988) achieved 88% triploids by treating fertilized *C. nobilis* eggs with 0.5 mg/l cytochalasin B in

0.01% DMSO commencing 15 minutes post-activation for 15 minutes duration at a temperature of 25°C. Komaru and Wada (1989) report production of 23% triploids using pressure (200 kg/cm<sup>2</sup>) as well as CB (75%).

Komaru et al. (1988) also undertook a comparison of three methods of ploidy determination; flow cytometry, DNA microfluorometry with DAPI staining and chromosome counting. The microfluorometric procedure, earlier used by Naruse et al. (1985) to determine the ploidy of fish larvae, was found to be a simple and useful method for quantification of DNA content; a valuable alternative to flow cytometry.

#### *Physiology*

Neither ripe eggs nor spermatozoa were observed in triploid scallops at a time when their diploid counterparts were spawning (Komaru and Wada 1989), and both shell width and tissue weight were greater in triploids than in diploids.

#### *Chlamys varia* (Black Scallop)

#### *Triploidy*

High percentages of triploid pediveligers of black scallop have been produced using CB (Baron et al. 1989). Comparison of diploid and triploid larvae revealed no significant difference in growth rate, however, mortality among triploids prior to metamorphosis was higher than for diploids over the same period (Baron et al. 1989).

#### *Pecten maximus* (Great Scallop)

#### *Triploidy*

Low percentages of triploids (up to 30%) have been induced using cytochalasin B in *P. maximus* (Beaumont 1986). Larvae were not reared beyond the early veliger stage, but analysis of early development indicated that, although larval yield was significantly reduced by CB treatment, this chemical did not significantly influence numbers of abnormally developing larvae. Both chromosome counts and polar body counts were used to determine percentage of triploid zygotes at the 3 hour stage. Results agreed reasonably well between methods, but polar body counts were easier and quicker to obtain.

One problem associated with triploidy induction in scallops is that certain species are hermaphroditic. Both eggs and sperm are often released together, making it difficult to control the timing of the maturation divisions. Furthermore, even a small proportion of self-fertilization may produce inbreeding depression which can be manifested in reduced growth rates (Beaumont and Budd 1983).

#### *Mytilus edulis* (Common Mussel, Blue Mussel)

#### *Triploidy*

Triploidy has been induced in *M. edulis* by both chemical and thermal methods (Yamamoto and Sugawara 1988,

Beaumont and Kelly 1989). Cytochalasin B targeted at meiosis I and meiosis II produced between 46% and 67% triploidy in Beaumont and Kelly's (1989) trials, but these authors were unable to produce similar percentages of triploids using heat shock. In contrast, Yamamoto and Sugawara (1988) reported yields of triploids in the range 70%–98% produced by heat shock treatment. Such discrepancies do occasionally occur between other studies and may stem from differences in broodstock condition, ambient temperatures, or handling techniques. In this particular instance, however, Yamamoto and Sugawara report a 2–22% incidence of triploidy amongst controls, a condition seldom observed (or at least seldom reported!) for this or other species. Yamamoto and Sugawara (1988) also experimented with cold shock. Recently, Yamamoto et al. (1990) reported increased yields of triploid *M. edulis* using a combination of heat shock (29°C) and caffeine (15 mM) (or 0.1 M CaCl<sub>2</sub>) compared to heat shock alone.

#### *Gynogenesis*

Activation of *M. edulis* eggs by UV-irradiated sperm is currently being studied at our laboratory (Fairbrother and Beaumont unpub. data). Preliminary results suggest that suitably UV-irradiated sperm will activate eggs to undergo their maturation divisions with the production of haploid embryos. However, as is the case for fish (Purdom 1983), all haploid embryos develop abnormally.

#### *Physiology*

Growth rate of putative triploid larvae from meiosis I, and meiosis II treated eggs were measured and compared with diploid controls by Beaumont and Kelly (1989). They report a significant increase in mean shell length of 36-day-old larvae derived from eggs treated at meiosis I compared to either control or meiosis II larvae, and suggest that this may be due to the postulated higher heterozygosity of meiosis I triploids. This experiment has since been repeated (Fairbrother and Beaumont, unpub. data) and has again demonstrated an enhanced growth rate of meiosis I triploid larvae.

#### *Mercenaria mercenaria* (Hard-shelled Clam)

#### *Triploidy*

Triploid hard-shelled clams have been produced by treatment with cytochalasin B (Buzzi and Manzi 1988, Hidu et al. 1988), but in contrast to other species, three-year-old triploid clams were found to have significantly lower dry weight and smaller shell dimensions than diploid controls (Hidu et al. 1988).

#### *Mulinia lateralis* (Dwarf Surf Clam)

#### *Triploidy and Tetraploidy*

Rupright (1983), using cytochalasin B to induce triploidy in the dwarf surf clam, found the mean live weight

of treated groups was significantly higher than that of controls. Scarpa (1985), has produced both triploid and tetraploid two-cell-embryos by targeting, meiosis II and first cleavage respectively. He also observed occasional instances of spontaneous triploidy in normal diploid groups.

#### *Parthenogenesis and Gynogenesis*

Scarpa (1985) produced egg activation, without the use of sperm, by the addition of 0.5–1.0 M KCl to an oocyte suspension for 8 minutes. Both maturation divisions occurred, but no cleavage was evident. Further attempts to produce KCl activated parthenogenetic diploids using CB, or combined heat and caffeine, to target meiosis II were unsuccessful and resulted in abnormal, uncleaved eggs (Scarpa et al. 1991, submitted).

Attempts to produce gynogenetic haploids using UV light irradiated sperm were more successful than the parthenogenesis trials. Eggs activated by UV treated sperm yielded presumed haploid embryos which, at 80 minutes, were one cleavage round behind those activated by normal sperm, and contained a high proportion of asynchronously dividing individuals compared to controls. Furthermore, Scarpa was able to produce diploid gynogens by CB induced "chromosome-doubling" at either meiosis II or first cleavage. Zygotes reached the two cell stage by 80 minutes post-activation, but failed to survive beyond 24 hours.

#### *Mya arenaria* (Soft-shelled Clam)

##### *Triploidy*

Cytochalasin B has been used to induce triploidy in *M. arenaria*, (Allen et al. 1982, Allen 1983, Allen et al. 1986b, Allen 1987a, Mason et al. 1988).

##### *Physiology*

###### (i) *Gonadogenesis*

A detailed study of gametogenesis in triploid soft-shell clams was undertaken by Allen et al. (1986b). None of the triploids they examined could be definitely classed as males. Females, however, could be identified and often showed a proliferation of pockets of oogonia with no apparent differentiation into oocytes.

###### (ii) *Growth Rate*

According to Mason et al. (1988), triploid clams produced by CB treatment at meiosis I, weighed slightly less than diploids of the same length, and, as triploid clams increased in body weight, their shells tended to be less inflated than those of diploids of equal size. Few significant differences between diploid and triploid juvenile clams were found with respect to energy budget components. However, seven loci were electrophoretically assayed and revealed triploid individuals to be nearly twice as heterozygous as their diploid siblings. Triploid variances were less

than diploid variances for every variable measured, which is what might be expected since high heterozygosity has been shown to correlate, in general, with reduced variance of polygenic traits (Zouros and Foltz 1987). However, the higher heterozygosity of triploids is not manifested as higher growth rate compared to diploids. Significant positive correlations between multiple locus heterozygosity and growth rate have, nevertheless, been documented for many bivalves (Zouros and Foltz 1987).

#### *Tapes philippinarum* (Manila Clam)

(= *Ruditapes philippinarum*; = *Tapes semidecussatus*)

##### *Triploidy*

High percentages (70–80%) of triploids have been produced using CB treatment in this species (Beaumont and Contaris 1988, Gosling and Nolan 1989, Dufy and Diter 1990). Gosling and Nolan (1989) have also successfully produced triploids by heat shocks but with lower yields.

##### *Tetraploidy*

Diter and Dufy (1990) succeeded in producing tetraploid embryos by chemical targeting, either during early meiosis I or just before first cleavage. Up to 65% tetraploid embryos were produced but none were detected in four-month-old spat. These results show a similar trend to those obtained by Stephens and Downing (1988) and Cooper and Guo (1989) working with *C. gigas*.

##### *Survival*

As with most other species, there is clear evidence that the use of CB reduces the initial survival of triploid groups compared to controls (Beaumont and Contaris 1988, Dufy and Diter 1990, Diter and Dufy 1990). Differential mortality of triploids and of tetraploids during larval development is also evident (Dufy and Diter 1990, Diter and Dufy 1990).

#### *Haliotis discus hannai* (Pacific Abalone)

##### *Triploidy*

Both hot and cold shocks have been used successfully to induce triploid abalones (Arai et al. 1986, Fujino et al. 1987, Fujino et al. 1988a, 1988b), with best percentages ranging from 60% to 87%. The use of pressure to induce triploidy has also proved successful, Arai et al. (1986) having produced 60% triploids by this method.

##### *Gynogenesis*

Eggs of the Pacific abalone have been successfully activated by ultra-violet light irradiated sperm leading to haploid and aneuploid larvae (52 hrs) (Arai et al. 1984), but diploidization techniques were not applied. More recently, Fujino et al. (1990) have produced gynogenetic diploids by targeting meiosis II.

### *Survival*

Fujino et al. (1987) have examined the relationship between high and low temperature stress tolerance and ploidy. Comparison of high temperature tolerance in diploids and triploids showed greatest survival in meiosis I triploids, followed by diploids with lowest survival in meiosis II triploids. Survival appears to correlate with high homozygosity for particular thermo-resistant alleles, rather than with any potential increase in heterozygosity present in triploids.

### *Recombination Frequencies*

It is possible, using electrophoretic analysis of meiosis II gynogenetic diploids, or triploids, from single parent matings, to estimate recombination frequencies at gene loci (Thompson and Scott 1984, Allendorf and Leary 1984). Fujino et al. (1988a, 1988b) have attempted similar analyses in triploid abalone. However, their data were complicated by their use of multiple-parent matings, necessitating error-prone estimations of both the proportional gametic contribution of each parent, and the differential survival of each potential offspring cross, before recombination frequencies could be calculated.

### SUMMARY AND CONCLUSIONS

The last decade has seen considerable progress in the development of ploidy manipulation techniques in molluscan shellfish. Cytochalasin B (CB) has proved to be a reliable chemical agent for inducing "chromosome-doubling" during the maturation divisions in all species. Thermal or pressure shock can also be used but, generally, with a lower success rate. This is probably because the timing of physical shocks is more critical; eggs have only a small window of vulnerability compared to chemical treatment.

Although CB generally produces higher percentages of triploids than other treatments, it also causes higher percentages of abnormalities and increases mortality during early development. From a commercial point of view this is not usually a problem, because molluscan shellfish are so fecund that very high initial mortalities can be tolerated without endangering commercial viability. Concern has been expressed in some countries (eg. Ireland; Gosling and Nolan 1989) about the hazard to humans associated with the use of CB, which is highly toxic, but its use on a commercial basis has been approved in the USA (Allen 1988a).

Triploid shellfish can be produced by targeting either meiosis I or II, but the genetic consequences of these two alternatives are different. There is some evidence, based on electrophoretic analysis, which demonstrates higher heterozygosity in meiosis I triploids and there are also growth rate data which suggest that meiosis I triploid larvae may grow faster than meiosis II triploids or diploids. However the potential theoretical advantage of higher heterozygosity in meiosis I triploids has yet to be convincingly demonstrated.

This potential advantage has not been exploited during the commercial development of triploid induction in oysters, where it is always meiosis II which is targeted (Allen et al. 1989). However, one reason may be that, in general, a higher percentage of triploids with lower mortality is obtained by targeting meiosis II rather than meiosis I.

Research has confirmed that triploid shellfish are essentially sterile, although some gonad will develop in both males and females. An interesting and unexpected observation is the large increase in hermaphrodites amongst triploid oysters, compared to diploids. Triploid oysters have already proved their worth as a commercial product (Allen et al. 1989); furthermore, the potential use of triploid shellfish in the study of the physiology and endocrinology of gonadogenesis, and also genetic and physiological aspects of heterosis, is now emerging.

Although commercial triploidy induction is now routine for oysters, attempts to induce viable tetraploids have not been successful in any of the molluscan shellfish species. It is not clear how, under certain conditions, tetraploids are produced during treatment which is designed to produce triploids. Nor is it understood why all such tetraploid zygotes fail to develop, even to the veliger stage. Tetraploidy induction based on "chromosome-doubling" during first cleavage has also been singularly unsuccessful in molluscs (though not in fish, Chourrout 1984). Again the reason is not known, but we wonder whether the 180° switch of spindle orientation between the first two cleavage divisions may be important in preventing the successful alignment of chromosomes on the second cleavage spindle after treatment.

There is little published evidence that techniques for producing diploid gynogenetic molluscs have been successful although, in the long term, the potential is there for the production of shellfish with "hybrid vigour" by crossing inbred gynogenetic lines. In the meantime, diploid gynogenesis offers the potential to provide highly homozygous organisms for genetic studies.

Several techniques are now available for assessing ploidy. Chromosome counting is rather intricate and time consuming, and flow cytometry is rapid and effective, but depends on access to a flow cytometer. Microfluorometry with DAPI staining and nuclear sizing on a Coulter counter also require access to expensive equipment, but nuclear sizing with an eyepiece graticule on an ordinary microscope might provide a cheaper, more effective, alternative.

There is little doubt that, with the current technology available, and the increasing importance of cultured shellfish as a world food source, development of ploidy manipulation in shellfish will continue to expand in the next decade.

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## CONSUMERS AND "EXPERTS" ALIKE PREFER THE TASTE OF STERILE TRIPLOID OVER GRAVID DIPLOID PACIFIC OYSTERS (*CRASSOSTREA GIGAS*, THUNBERG, 1793)

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**ABSTRACT** Oyster consumption in many regions of the U.S. drops significantly in the summer when oysters are gravid and soft. Sterile triploids produce less gonad and have higher glycogen than diploids and therefore may taste better. We ran sensory evaluations to test the acceptance/preference of triploid oysters in two panels: consumers and oyster growers ("experts"). Oysters were rated for flavor, texture, and overall preference. The mean ratings by the two panels for the three sensory categories were nearly identical. In both sets of ratings, triploids were rated superior to control diploids probably because they had higher levels of glycogen. The firmer, sterile triploids were rated higher for texture than softer, gravid diploids. Both panels significantly favored triploid oysters over diploids in overall preference. Scores for diploids were more variable than those for triploids, suggesting decreased variability in the quality of the product might be an additional attribute of sterile oysters.

**KEY WORDS:** *Crassostrea gigas*, triploid, consumer, sterility, taste test

### INTRODUCTION

Traditionally, oyster (*Crassostrea gigas*) sales in Washington state drop by 50% or more during the summer, i.e., those months not containing "R's." During this time, oysters are undergoing sexual maturation which reduces their marketability. Oyster consumption declines because gametogenesis alters the meat texture. Oysters, firm in the winter, turn soft and milky in the summer; following spawning, they become watery. Further, the sweet taste is reduced as stored glycogen reserves are utilized to make gametes (Gabbott 1975, Mann 1979). The oyster industry would like to produce a quality product year-round because production of a firm "summer oyster" could result in a 35% increase in sales for Pacific northwest growers alone (Breese and Malouf 1977).

Our approach to producing a summer oyster was by inducing triploidy using chromosome set manipulation. Triploids contain an extra set of maternal chromosomes retained by the egg when treated with chemical or physical agents shortly after fertilization (Downing and Allen 1987). Triploids are sterile and usually devote less effort to reproduction than fertile diploids. For example, triploid soft-shell clams (*Mya arenaria*), bay scallops (*Argopecten irradians*), Pacific and American oysters (*C. gigas* and *C. virginica*), and Japanese scallop (*Chlamys nobilis*) produced significantly less gonad than their diploid counterparts (Allen et al. 1986, Tabarini 1984, Allen and Downing 1990, Lee 1988, Komaru and Wada 1989). In addition, glycogen levels were found to be significantly higher in triploid bay scallops and Pacific oysters (Tabarini 1984, Allen and Downing 1986). Does reduced gonad production

and higher glycogen content produce a tastier product? We conducted two sensory evaluations to test acceptance/preference of triploid oysters by consumers and oyster growers. Consumers rated oysters that were lightly steamed and oyster growers compared oysters on the half-shell.

### METHODS AND MATERIALS

#### *Experimental Oysters*

Oysters used in this study were produced as part of a larger study on induced triploidy in the Pacific oyster, *C. gigas*, using the chemical cytochalasin B (Downing and Allen 1987). Cytochalasin B (approved for treating oyster eggs according to our procedure [ibid.] by the U.S. Food and Drug Administration) produced triploids by preventing polar body extrusion. Oysters used in this study were produced in May 1984: 3.5 million fertilized eggs were treated with 1 mg cytochalasin B (previously dissolved in 1 ml dimethylsulfoxide) in 1 L of seawater held at 18°C. The treatment lasted from 15–30 minutes after fertilization. After treatment, eggs were sieved onto 25 µm Nytex screen and resuspended in 0.1% dimethylsulfoxide in seawater for 15 minutes. Larvae were reared in standard hatchery fashion and set on oyster shell cultch.

Following metamorphosis and settlement, the cultch was strung on ropes according to treatment group. We sampled spat at 1 month and determined the proportion of triploids using flow cytometry (see Downing and Allen 1987 for technique). During August 1984, oysters were planted on experimental plots located in Oakland and Mud Bays in southern Puget Sound.

#### *Preparation of Oysters for Consumer Panel*

On August 14, 1985, the day prior to our test, 82 oysters were collected from Oakland Bay, Washington. The ploidy of each oyster was determined by removing the top shell of

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each oyster and preparing a small biopsy of the adductor muscle for flow cytometry. Oysters were covered to prevent dehydration and held on ice. Of the 82 oysters, 40 were triploid. After ploidy determination (which took ~2 hr) the meats of diploids and triploids were removed from their shells and placed in jars according to ploidy. Total weights of the meats was taken to demonstrate that, in general, diploid and triploid oysters were the same size (~13 g each oyster).

The consumer panel was administered by the Utilization Research Division (URD) of the National Marine Fisheries Service (NMFS) in Seattle, Washington and was held in their sensory evaluation facility. Consumers ( $N = 32$ ) were mainly volunteers from the NMFS staff, representing both sexes equally. Individuals sat in separate booths. Each booth contained a plate with the oysters, an evaluation sheet and a pencil, etc.

Immediately before testing, the oysters were cooked in the NMFS URD kitchen. Oysters were placed inside aluminum pans, and the lids were marked A for triploids or B for diploids. Pans were then placed together in a large tray over boiling water, and the oysters were steamed for 4 minutes. Triploid and diploid oysters were removed and placed accordingly onto white plates labelled A and B with tape. The plates were then delivered to the panelists at the booth. Each consumer rated oysters labelled A and B for flavor, texture, and overall preference. The first two sensory categories were rated on a 5-point scale from poor [1] to very good [5]. Preference was rated on a 9-point hedonic scale from dislike extremely [1] to like extremely [9]. There was also a comment column.

#### *Preparation of Oysters for "Expert" Panel*

On August 27, 1985, oysters were collected from Mud Bay, Washington. Analysis of ploidy was done as before except the top shell was replaced and held in place with a elastic band to preserve freshness. The oysters were kept on ice until the next day when they were served raw to attendees of the August meeting of the Pacific Oyster Growers Association. We also served oysters provided by a gourmet half-shell producer, as a reference oyster. These gourmet diploid oysters were grown in Wescott Bay (located in the San Juan islands) by Wescott Bay Sea Farms; they were not part of our experimental production of May 1984. The object of the gourmet diploid was to provide each taster with the best oyster available in this season to serve as a hallmark of quality (a reference). Reference oysters were opened at the meeting and were so noted to all panel members. Previously opened triploid and diploid oysters were re-opened and labelled B and C, respectively (reference oysters were labelled A). This evaluation was held in a meeting room at a local motel and was administered by the authors. The setting was less formal than at NMFS because panelists sat at long tables and individuals were not partitioned from each other. Otherwise the setting

approximated evaluation booths. Conversation was discouraged. Members of the association were assumed to be more experienced oyster eaters than the previous panel, but none were trained in sensory evaluation. Nineteen members rated the three oysters for texture, flavor, and overall preference using the scales previously described.

#### *Experimental Design and Statistical Analysis*

A complete randomized block design was run in which each panelist formed a block with one replicate of each type of oyster. Wilcoxon's paired-sign test was used to compare the triploid and diploid scores from the Consumer Panel. Wilcoxon's was also used to compare scores of diploids grown in different bays from the "Expert" Panel (Steel and Torrie 1980). Data from the "Expert" Panel were analyzed using Friedman's test, a non-parametric approximation of ANOVA. Rejection of the null hypothesis that types of oysters did not differ was done at  $p < 0.05$ . A non-parametric equivalent to Student-Newman-Keul's test was done when a null hypothesis was rejected by Friedman's test (Zar 1974).

## RESULTS AND DISCUSSION

We assume that the properties distinguishing diploids from triploids are similar in both raw and cooked oysters, as long as both diploids and triploids were grown in the same environment. Below we compare ratings for flavor and texture (a) between diploids and triploids within bays and (b) among bays for diploids. These comparisons are conservative because panelists were not trained in sensory evaluation. Cardello et al. (1982) has shown that with training consumers improved their abilities to distinguish subtle taste differences.

#### *Flavor*

Both panels rated diploids from Oakland and Mud Bays similarly with a mean score of 3.4 ("fair"); reference diploids (grown in Wescott Bay and scored in the growers panel only) and triploids rated ~4.0 ("good") (Table 1). The consumer panel overwhelmingly preferred the flavor of triploid to diploid oysters (Wilcoxon's paired-sign test;  $T = 54$ ,  $p < 0.001$ ).

"Experts" could not distinguish a difference in flavor among the three types of oysters (Friedman's test;  $X^2 = 3$ ,  $p = 0.22$ ). However, triploid oysters from Mud Bay were rated more flavorful than diploids from the same bay (Wilcoxon's  $T = 22$ ,  $p < 0.05$ ). Possibly, glycogen content was a factor contributing to the significant difference in flavor between diploids and triploids. Oysters store carbohydrates in the form of glycogen, which contributes to their sweet taste (Hughes-Games 1977). Glycogen content normally varies on an annual cycle: high in the winter and low in the summer when stored glycogen is used to produce gametes (Gabbott 1975). We assume that diploid oysters were following this annual cycle and consequently had rel-

TABLE 1.

Mean ratings (standard deviation) for three sensory categories: flavor, texture, and overall preference. Evaluations by the two panels were done on scales from 1–5 for flavor and texture, and from 1–9 for overall preference. Oysters used in the tests were grown in various locations [in brackets]: M = Mud Bay and O = Oakland Bay (Puget Sound); W = Wescott Bay (San Juan islands). Levels of statistical significance: \* =  $p < 0.05$ ; \*\*\* =  $p < 0.001$ .

Category	Consumer	"Expert"
Flavor		
Gourmet diploid		3.9 (0.71) [W]
Diploid	3.4 (1.22) [O]	3.4 (1.01) [M]
Triploid	4.2 (0.77) [O]***	3.9 (0.77) [M]
Texture		
Gourmet diploid		3.5 (1.0) [W]
Diploid	3.3 (1.14) [O]	3.2 (1.14) [M]
Triploid	4.1 (0.71) [O]***	4.0 (0.89) [M]
Overall Preference		
Gourmet diploid		6.7 (1.45) [W]
Diploid	5.8 (2.26) [O]	6.4 (1.61) [M]
Triploid	7.5 (1.26) [O]***	7.4 (1.26) [M]*

atively low glycogen levels at testing time. In contrast, sterile triploids maintain most of their glycogen stores during periods of gametogenesis (Allen and Downing 1986), probably accounting, in part, for their higher flavor rating.

Reference oysters from Wescott Bay were rated by "Experts" equally as flavorful as triploids. There are a number of possible reasons for this. Wescott Bay is a more productive bay than Mud Bay and, for suspension feeding oysters, environment is a factor contributing to flavor (Ventilla 1984). Broodstock from Wescott Bay were selectively bred for high glycogen content (Perdue 1983). And of course, reference oysters also may have been fresher, having been opened immediately prior to tasting. It is interesting that triploids grown in a less productive bay scored as highly as diploids grown in a more productive one.

#### Texture

The mean rating for texture in triploids, regardless of the bay of origin, was higher than for diploids (Table 1). Diploids, which ranked similarly on average in all three bays, scored "fair" (3) and triploids were "good" (4). The texture of triploids was preferred in the Consumer panel (Wilcoxon,  $T = 45$ ;  $p < 0.001$ ) but in the "Expert" panel, preference could not be demonstrated statistically (Friedman's  $X^2 = 3.7$ ;  $p = 0.16$ ). Sensory evaluations were conducted during the period when gonad was near

maximum in diploids, so the difference in meat quality between gravid diploids and sterile triploids would have been greatest. In previous studies, we found that sterile triploids produce only 25–40% of the gonad compared to diploids (Allen and Downing 1986). We conclude that the extent of gonad maturation is responsible for the reduced ratings for texture. Eggs and sperm, high in lipid and moisture content, impart a "milky" consistency to diploids (Perdue 1983). Triploids remain firm throughout the summer (Allen and Downing 1986, 1990).

#### Overall Preference

Both panels rated triploid oysters significantly higher than diploids for overall preference (Wilcoxon  $T = 52$ ;  $p < 0.001$  and Friedman  $X^2 = 6$ ;  $p < 0.05$ ) (Table 1). Scores for diploids corresponded to approximately 6, "like slightly." Those for triploids were approximately 7.5, "like moderately" to "like very much." The overall rating for preference in the diploids does not seem low enough to be commensurate with the large decline in consumption usually observed during the summer. One reason might be that the test oysters were yearlings, therefore were less gravid than older oysters would be. We also observed that variances in all taste categories (except flavor for the reference oyster) were greater for diploids than for triploids. Our histological studies indicate that only half of diploid yearlings undergo substantial gametogenesis (unpublished data). Variation in extent of gametogenesis in diploids seems to correspond with variance in sensory categories. Perhaps product variability discourages sales during the summer as much as unacceptable taste. Production of sterile oysters that are consistent in texture and flavor year-round should help maintain sales during the summer months, perhaps putting to rest the axiom, "Don't eat oysters in the months without R."

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## FACTORS AFFECTING THE SHELF LIFE OF LIVE CULTURED MUSSELS

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**ABSTRACT** Efficient marketing of live mussels through retail outlets necessitates knowledge of their tolerance to aerial storage. Several factors thought to affect shelf life and weight loss during storage were examined. A comparison of intertidally and subtidally grown *Mytilus galloprovincialis* and subtidally grown *Choromytilus meridionalis* failed to show differences in survivorship during aerial storage although *M. galloprovincialis* lost less weight during storage. Three aspects of post-harvest handling, namely storage temperature, the use of plastic bags for packaging, and conditioning by rewatering after sorting, were also examined. Survival was enhanced at reduced storage temperature, but temperature had little effect on rate of weight loss. Storing mussels in plastic bags substantially reduced weight loss, but did not affect survival. Mechanical sorting had a negative effect on subsequent survival, but conditioning by rewatering allowed mussels to recover. Survival rates increased with duration of rewatering, with 12 to 24 hours giving the best survival during storage for the least mortality during the rewatering process. The optimal procedure is thus to condition mussels for 12 to 24 hours before packaging them in plastic bags and storing them at 4°C during shipment and in storage.

**KEY WORDS:** mussels, storage, aerial exposure

### INTRODUCTION

Four mussel species are abundant along the southern African coast. Of these, three—*Aulacomya ater*, *Choromytilus meridionalis* and *Perna perna*—are indigenous, while the fourth, *Mytilus galloprovincialis*, is believed to be a recent introduction (Grant et al. 1984, Grant and Cherry 1985, Grant 1987, van Erkom Schurink and Griffiths 1990). Subsistence exploitation of these species is a traditional but rapidly escalating activity, especially along the Transkei and Natal coasts (DeFreitas and Martin 1988, Hockey et al. 1988, van Erkom Schurink and Griffiths 1990). By contrast, mussel culture is a recent innovation in the area. The first farm, based mainly on the introduced *M. galloprovincialis*, came into production in 1984. Since then, output has increased dramatically to around 800 tons per annum from four farms in 1989. Much of this production is canned or frozen, but a substantial proportion is sold live, largely in population centres far removed from the farms. Thus, for economic and public health reasons, farm managers and retailers wish to optimise the shelf life of aerially stored live mussels.

A number of parameters may be manipulated in order to prolong shelf life. These include the selection of appropriate culture species and the culture techniques used—such as whether mussels are cultured inter- or subtidally (Dare 1974). Postharvest handling is also important (Dare 1974, Slabyj 1980, Warwick 1985), as is the temperature of storage (Slabyj 1980, Warwick 1985) and the type of storage container. Conditioning, or rewatering, of mechanically sorted mussels prior to storage is also known to enhance subsequent survival (Slabyj 1980). This paper aims to investigate and quantify the effects of the above variables on the shelf life of South African cultured mussels.

### METHODS

#### *Effects of Culture Techniques*

Shelf life of three groups of mussels was compared. These were subtidally and intertidally grown *M. galloprovincialis* and subtidally grown *C. meridionalis*. Immediately after harvest mussels were returned to the laboratory for storage and monitoring. Individuals of similar size (60–70 mm) from each of the three categories were subdivided into six trays holding 50 mussels lying on their sides. Pairs of trays from each category were then stored in constant temperature rooms at 4, 10 and 18°C, respectively. One tray from each pair was left open to the air and the other enclosed in a plastic bag. A group of 10 mussels from each tray were individually marked and their whole wet weights recorded daily with a top loading balance accurate to 0.01 g.

All mussels were checked daily for mortality. Death was assumed to have occurred when mussels were unable to hold the two shell valves closed. Since live mussels are known to gape (Coleman and Trueman 1971, Coleman 1973, Dare 1974, Ameyaw-Akumfi and Naylor 1987), the valves of gaping mussels were squeezed closed for five seconds and then released. If reopening was immediate death was assumed to have occurred, but if the valves remained closed, or if reopening was only partial the mussels were assumed to be alive.

#### *Effects of Post-Harvest Handling*

Two further experiments were carried out to determine the effects of mechanical sorting and conditioning (rewatering) on the survival of subtidally cultured *M. galloprovincialis*. Mussels of 60–70 mm shell length were har-

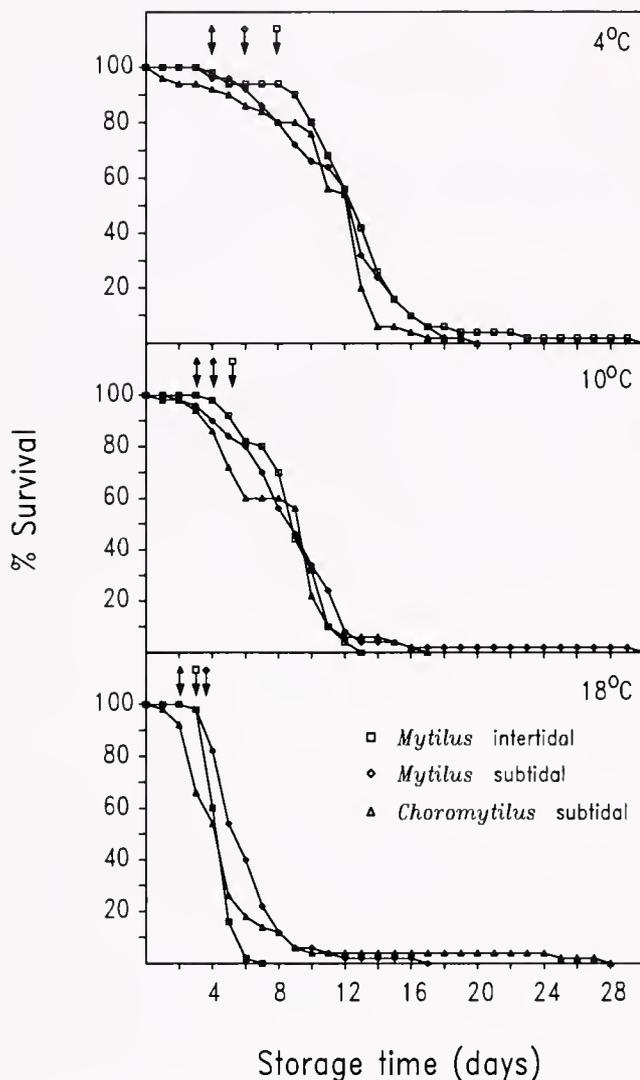


Figure 1. Percent survival of intertidally and subtidally grown *Mytilus galloprovincialis* and subtidal *Choromytilus meridionalis* stored at 4, 10 and 18°C. Arrows indicate time to 10% mortality.

vested from longline ropes and divided into three equal batches. Two of these were subjected to mechanical sorting in a wire drum device as described by Dare (1974), while the third was gently declumped by hand (hereafter referred to as handsorted mussels). Of the two mechanically sorted batches one was stored at 4°C directly after sorting, while the other was placed in a flow-through rewatering tank of approximately 3000 l capacity for 7 days before being stored in the same way. The flow rate through the tank was 600 l min<sup>-1</sup> and the water temperature 15°C, the same as that in the culture area.

The second experiment examined the effects of the duration of conditioning in greater detail. Cultured *M. galloprovincialis* were rewatered for periods of 1, 2, 4, 6, 12, 18, 24, 48, 72 and 96 hours after mechanical sorting. Mortality during the rewatering process was recorded and only mussels alive after rewatering were transferred to the trays.

Thereafter 50 mussels from each trial were stored exposed at 4°C and subsequent mortality rate monitored. Final yield was calculated as the percent of the original crop surviving both the rewatering process and subsequent storage.

## RESULTS

Comparisons of the survivorship of mussels grown under different culture techniques showed little differences (Fig. 1). Subtidally grown *M. galloprovincialis* survived as well as those grown intertidally, and *C. meridionalis* followed a similar trend. In contrast, temperature of storage had a profound effect on shelf life, with times to 10% mortality declining markedly at increased temperatures. Time to 10% mortality ranged from 5 to 9 days at 4°C to 3 to 5 days at 10°C and 2 to 3 days at 18°C. The rate of mortality, as given by the slope of the descending portion of the curve, was also found to increase with temperature.

Storing mussels in plastic bags had little effect on the survival of *M. galloprovincialis*, but did enhance that of *C. meridionalis* stored at 4°C, particularly following 10 or more days of storage (Fig. 2). Time to 10% mortality was, however, not affected.

Weight data (Table 1) clearly demonstrate a progressive decline in mass. Both species lost similar amounts of weight, and temperature of storage had little effect on the rate of weight loss. Mussels stored in plastic bags, however, appeared to lose less weight than those stored exposed (Table 1). One-way ANOVA and Tukey's multiple range test indicated no significant differences between exposed and bagged mussels after two days of storage. After five days significant differences ( $p < 0.05$ ) were observed in the 18°C treatment for both species and in the 10°C treatment for *C. meridionalis*. Bagged mussels lost significantly less weight than exposed ones at all temperatures after 10 days of storage.

The survival of *M. galloprovincialis* which were mechanically sorted was seriously impaired relative to controls manually removed from the culture ropes and placed directly into the storage trays (Fig. 3). Mussels which were conditioned by replacing them in a rewatering tank for 7 days after being sorted survived almost as well as handsorted mussels, and far better than sorted but unconditioned ones. Times to 10% mortality varied from 1 day for mechanically sorted, unconditioned mussels to 6 days for conditioned mussels and 11 days for handsorted mussels (Fig. 3).

Variations in the duration of conditioning were found to have a marked effect on survival (Fig. 4), the effects being particularly dramatic over the first 24 hours of conditioning. Although all groups showed between 85 and 100% survival after the first 2 days of storage, rewatering for as little as 12 hours increased survivorship at 5 days from 56% to 88%. After 10 days of storage less than 10% of unconditioned mussels remained alive, but this figure increased to 38% following only 6 hours of conditioning and to as much

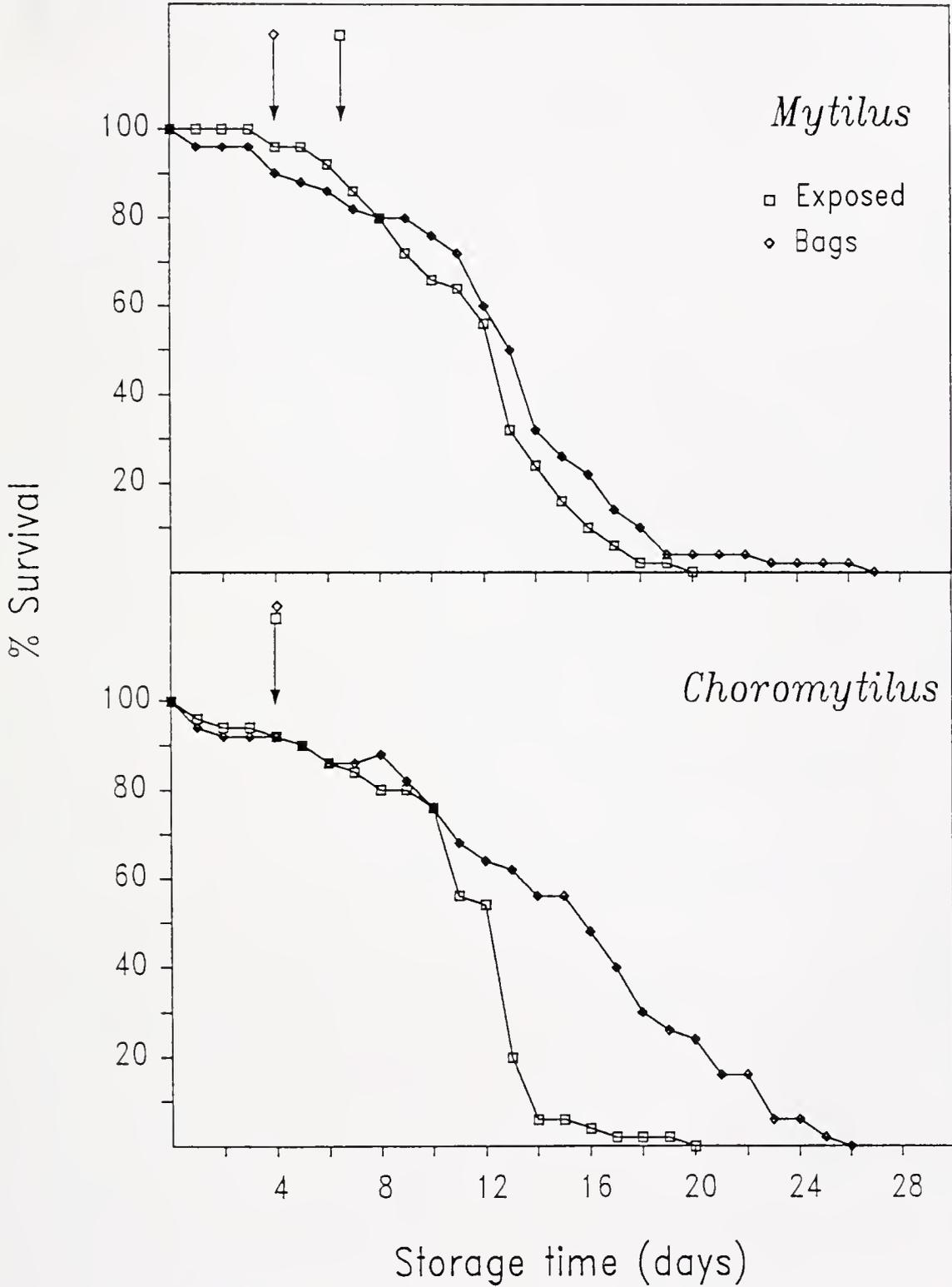


Figure 2. Percent survival of *Mytilus galloprovincialis* and *Choromytilus meridionalis* exposed and in plastic bags at 4°C. Arrows indicate time to 10% mortality.

TABLE 1.

Percent weight loss by *Mytilus galloprovincialis* and *Choromytilus meridionalis* after 2, 5 and 10 days of storage exposed and in plastic bags at 4, 10 and 18°C.

Days of Storage		<i>M. galloprovincialis</i>			<i>C. meridionalis</i>		
		2	5	10	2	5	10
Exposed	4°C	3	9	20	1	12	23
	10°C	2	8	16	2	11	19
	18°C	4	13	20	2	16	24
Bagged	4°C	1	5	7	4	7	12
	10°C	1	3	13	1	4	11
	18°C	4	12	13	4	5	10

as 78% for mussels conditioned for 48 hours or more. It is, however, important to note that mortality was associated with the conditioning process itself and this increased with duration of rewatering (Table 2). This mortality increased dramatically from 0–5% for periods of conditioning of less than 24 hours to 29–36% after 48 hours. The yield, or percent of the original crop surviving after 5 days of storage, was calculated from the percent mortality during rewatering and that of mussels stored for 5 days (Table 2). Instead of increasing with duration of rewatering, this composite sur-

vival peaked at between 84 and 91% when mussels were rewatered for periods of 12 to 24 hours prior to storage.

## DISCUSSION

### Effects of Culture Techniques

The survival of subtidal and intertidal *M. galloprovincialis* in storage did not differ substantially (Fig. 1). This is contrary to the findings of Dare (1974) who suggested that intertidal mussels survived better during aerial storage than subtidal ones. However, the greater survival potential of Dare's mussels was not great enough to justify culturing mussels in the intertidal zone, where growth rates are greatly reduced (Griffiths 1981, Griffiths and Griffiths 1987). On the other hand, the present study illustrates that subtidally cultured mussels are just as hardy as their intertidal counterparts, despite their lack of experience of aerial exposure.

Although *C. meridionalis* is not favoured for culture because of the dark brown colour of the female gonad (van Erkom Schurink and Griffiths 1990) this species seeds itself onto longline ropes in vast quantities. Moreover, since it is easily confused with *M. galloprovincialis*, the two species are inevitably grown in mixed culture. Our survival studies show little difference in the ability of the two forms

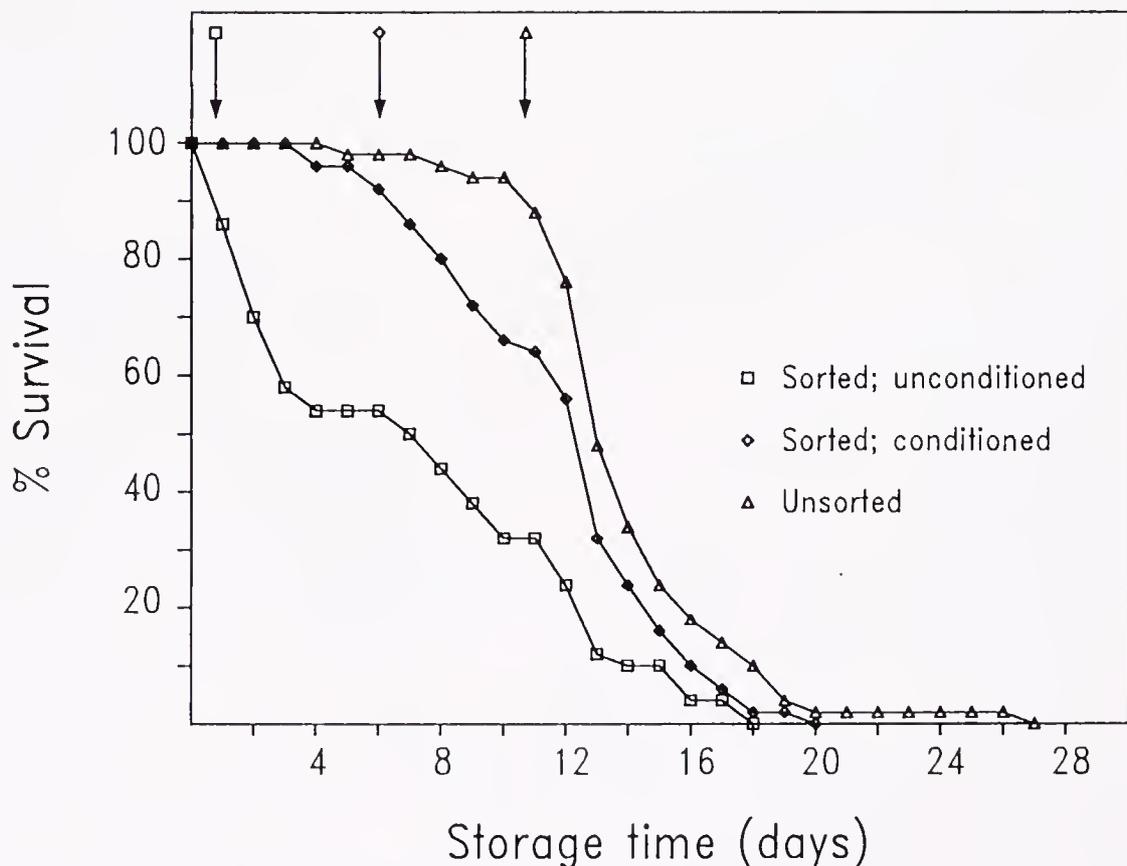


Figure 3. Percent survival of handsorted, sorted unconditioned and sorted conditioned *Mytilus galloprovincialis* stored 4°C. The arrow indicates time to 10% mortality.

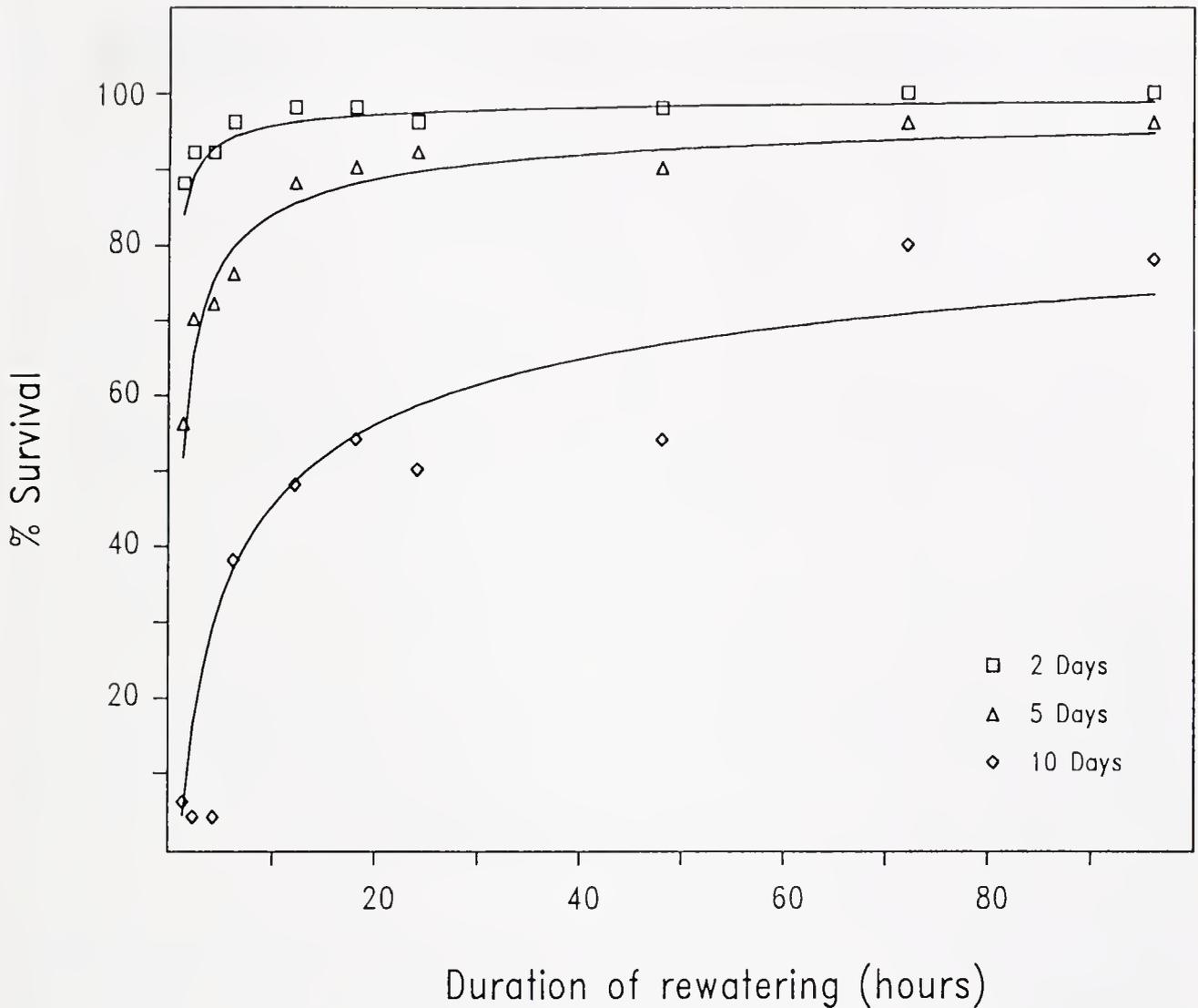


Figure 4. Percent survival of *M. galloprovincialis* at 2, 5 and 10 days of storage at 4°C after rewatering for different time periods.

to tolerate storage (Fig. 1), indicating that both could be transported live to distant markets once they gain acceptance from consumers. Weight loss data, however, show that *M. galloprovincialis* lost less weight than *C. meridionalis*. This difference in weight loss may be attributed to differential gaping of the two species and hence loss of water both from the mantle cavity and by drying of the flesh. Various authors (Coleman and Trueman 1971, Coleman 1973, Ameyaw-Akumfi and Naylor 1987) have found that the incidence of gaping of exposed mussels is related to their natural position on the shore and not to endogenous rhythms. Thus typically mid-shore species such as *M. galloprovincialis* would be expected to gape little, while species naturally occurring subtidally or low in the intertidal zone, like *C. meridionalis* (van Erkom Schurink and Griffiths 1990), might be expected to gape extensively when exposed. Differential gaping may thus be responsible for the lower weight loss of *M. galloprovincialis*. The com-

mercial implications of this water loss depend on the marketing technique. Where mussels are sold by live weight any water loss will result in a loss of income. It is thus in the interests of farm managers that as much weight as possible be maintained. This becomes even more important when mussels are sold by flesh weight only, as is often the case when mussels are frozen or canned. In such a case initial loss of water held in the mantle cavity would not affect yield, but subsequent weight loss during storage may render the mussels less juicy and possibly tougher. These factors thus make *M. galloprovincialis* a more suitable species for live storage than *C. meridionalis*.

#### Effects of Post-Harvest Handling

An inverse relationship between shelf life of mussels and storage temperature has been noted previously (Dare 1974, Slabyj 1980, Warwick 1985) and is a recognised feature of most perishable crops. Our results further indicate

TABLE 2.

Percent mortality of *Mytilus galloprovincialis* during rewatering and after 5 days of storage at 4°C after rewatering for various periods of time. Yield is the percent of the original crop alive after 5 days of storage.

Rewatering Period (Hours)	Mortality during Conditioning	Mortality during Post-Conditioning Storage	Yield
1	0	44	56
2	1	30	69
4	0	28	72
6	1	24	75
12	4	12	84
18	5	10	86
24	1	8	91
48	29	10	64
72	36	4	61
96	36	4	61

that storage temperature had little effect on rate of weight loss (Table 1). Placing the mussels in plastic bags did not markedly affect survival (Fig. 2), but did reduce weight loss (Table 1). Because all mussels gaped and lost liquor, the reduced weight loss of bagged mussels presumably occurred because of reduced evaporative loss from gaping mussels.

It has been suggested that the mechanical sorting of cultured mussels causes internal damage which decreases subsequent survival (Dare 1974). Survivorship curves (Fig. 3) indicate that mussels stored directly after sorting have a markedly lower tolerance to aerial exposure than hand-sorted mussels. Conditioning by rewatering after sorting allows the mussels a "recovery period" to repair damage sustained during declumping and sorting, and mussels treated in this way survived far better than those that were unconditioned, a phenomenon previously noted by Slabj (1980).

Increasing the duration of rewatering resulted in a corre-

sponding increase in survival during storage (Fig. 4). However, mortality of mussels severely injured during sorting continued to occur in the rewatering tank and reached 30% when mussels were rewatered for 48 hours or more (Table 2). Figure 4 suggests that the first 24 hours of rewatering is the most critical, and that rewatering for longer periods has little further effect on subsequent survival, at least over the first 5 days. Furthermore, significant mortality occurs during the rewatering process itself. Thus calculating the percent survival of the original crop by incorporating mortality during both rewatering and storage (Table 2) indicates that peak survival (84–91%) occurs when mussels are rewatered for periods of between 12 and 24 hours. It is thus recommended that conditioning by rewatering be carried out and that the duration of rewatering should not be longer than 24 hours. Depending on the rewatering capacity available and anticipated time to market even shorter periods of rewatering may be adequate.

Although at present mussels leaving South African farms are placed in plastic bags, no attempt is made to chill them during transportation, the average duration of which is 20 hours (Phillip Steyn, Seafarm Inc., pers. comm.). Once the mussels arrive at their destinations they are refrigerated at approximately 6°C and stored for two to four days before consumption. Thus storage after transportation is at a favourable temperature, although this could be lower still. However, Slabj (1980) and Warwick (1985) have stated that the time between harvest and chilling may be critical, and thus cooling before or during transportation is advised for maximum shelf life.

#### ACKNOWLEDGMENTS

Thanks go to the staff of Atlas Seafarms in Saldanha Bay for their generous help and enthusiasm, and to Lesley Prochazka who assisted with field work. This work was supported by a post-graduate bursary from the Foundation for Research Development. A grant in aid of publication by the Editorial Board of the University of Cape Town is gratefully acknowledged.

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## FILTRATION RATE AND GROWTH IN THE BLUE MUSSEL, *MYTILUS EDULIS* LINNEAUS, 1758: DEPENDENCE ON ALGAL CONCENTRATION

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**ABSTRACT** Filtration rates in mussels *Mytilus edulis* were measured at different algal (*Rhodomonas baltica*) concentrations to identify the algal concentration range within which the filtration capacity is exploited. Between about  $2-6 \times 10^3$  cells  $\text{ml}^{-1}$  the filtration rate is high and constant for at least 8 h. At algal concentrations of  $1.5 \times 10^4$  cells  $\text{ml}^{-1}$  and higher the filtration rate gradually decreases after an initial period of maximal filtration rate. The pattern in reduction of filtration rate at different high algal concentrations is very similar and irrespective of algal concentration. The reduction in filtration rate as a result of reduced valve gape is interpreted as a reaction to satiation of the digestive system, or less specifically to adverse conditions. Calculations based on maximally growing mussels show that the growth potential may be realized at an algal concentration of about  $3 \times 10^3$  *R. baltica* cells  $\text{ml}^{-1}$  (equivalent to about  $1.4 \times 10^4$  *Phaeodactylum tricornutum* cells  $\text{ml}^{-1}$ ). It is presumed that the generally low growth rates obtained in laboratory studies on a pure algal diet, as compared to maximal growth rates in nature, may be due to the use of unnaturally high algal concentrations which lead to suboptimal conditions (valve closure, reduced metabolism and reduced biosyntheses/growth).

**KEY WORDS:** mussel, *Mytilus edulis*, filtration rate, growth

### INTRODUCTION

Filtration rate is a fundamental parameter in bioenergetic studies of suspension feeding bivalves. A vast number of methods and measurements of bivalve filtration rates in relation to factors such as ambient algal concentration and temperature have been carried out since the beginning of this century, but basic questions still cause controversy in the literature.

During recent years properties of the mussel *Mytilus edulis* pump have been assessed by Jørgensen et al. (1986a, 1988, 1990) and Jørgensen (1989). Parts of these studies were devoted to examination of the relationship between valve gape and filtration rate. It was found that filtration rate declines concurrently with reduced valve gape leading to a shortening of the gill axes and reduction in width of the interfilament canals. Presumably because interference between opposing bands of water pumping lateral cilia becomes negative with decreasing width of the interfilament canals the pump potential is reduced. The ill-defined relations between valve gape and filtration rate did not appear to be a mechanism in controlling water processing and thus food ingestion, but the reduction in filtration rate was found rather to be a secondary effect of the mussels' response to suboptimal conditions (Jørgensen et al. 1988). The absence of a physiological control mechanism of filtration rate as a means to control feeding rates in mussels is in conflict with the concept of energy optimization in mussels. An apparently inverse relationship between algal concentration and filtration rate in mussels has been interpreted as a regulatory mechanism which controls the filtration rate above a certain high algal concentration in such a way that the

number of cells filtered is kept constant (Winter 1973, Navarro and Winter 1982, Sprung and Rose 1988). Thus, it remains to be shown if reduced filtration rate at high algal concentrations is due to 'suboptimal conditions,' a protective reaction against overloading of the feeding system, as suggested by Riisgård and Møhlenberg (1979), or to a regulatory mechanism as assumed by Winter (1973) and Navarro and Winter (1982).

Many laboratory studies on growth and energetics in suspension feeding bivalves have been undertaken. Peak growth rates achieved in the laboratory at apparently optimal concentrations of algae are usually less than maximal growth rates observed in nature (Kiørboe et al. 1981, Jørgensen 1990). This may be an indication of the difficulties of creating optimal conditions in the laboratory, including identification of the food regime to which the bivalves are adapted. Growth rates comparable with those observed in nature may only be obtained in laboratory experiments when these are carried out at algal concentrations to which the bivalves are adapted. This condition seems to have been largely ignored (Jørgensen 1990).

In the present work filtration rates in mussels, *Mytilus edulis*, have been measured at different algal concentrations to identify the concentration range within which the filtration capacity is exploited. Further, the pattern in filtration rates above the critical algal concentration at which the mussels reduce the valve gape have been established, and the findings are discussed in relation to the calculated minimal algal concentration required for maximal growth as observed in nature. Finally, the possible adjustment of filtration rate of cold-adapted mussels to higher temperature

has been examined, supplementing results from a recent study on the temperature effect on the mussel pump (Jørgensen et al. 1990).

#### MATERIALS AND METHODS

The experiments were performed on *Mytilus edulis* L. collected at Fyns Hoved, Funen, Denmark, in February 1989 (5.9°C; 20‰ S) and transferred to an aquarium with running seawater (6.2°C) at the nearby Biological Station. During the following days the effect of temperature on filtration rate was measured in a group of 25 mussels. These data have been published (Jørgensen et al. 1990). The same group of 25 mussels (30.6 ± 0.7 mm shell length) was used in the present follow-up study. The aquarium with the mussels was transferred to a temperature-controlled water bath at the Biological Institute and kept at 15°C for 28 days. The filtration rate was then measured to see if the maximal filtration rate in cold adapted mussels (5.9°C) differed from the filtration rate capacity in mussels acclimatized to higher temperatures. Because the mussels spawned after 30 days at 15°C the temperature was lowered to 9°C to prevent further spawning. During the following 28 days the filtration rate at this temperature was repeatedly measured at low algal concentrations (2–4 × 10<sup>3</sup> cells ml<sup>-1</sup>) previous to measurement of the filtration rate as a function of exposure time to different high (1.5–4 × 10<sup>4</sup> cells ml<sup>-1</sup>) algal concentrations.

**Filtration rate.** The filtration rate was measured as the volume of water cleared of flagellate cells (*Rhodomonas baltica*, almost spherical cells, 5–6 µm in diameter). The particle size for optimum retention efficiency is about 4 µm in *Mytilus edulis* (Møhlenberg and Riisgård 1978). Flagellate cells were added to the strongly aerated aquarium with a known volume of water (V = 14.4 l) and the group of 25 mussels. The reduction in the number of particles as a function of time was followed by taking water samples every 5 or 10 min and measuring the particle concentration with an electronic particle counter (Elzone 80XY). Clearance (F) was determined from the exponential reduction in algal cell concentration as a function of time (always verified as a straight line in a semilog plot made by hand during the experiment) using the formula:  $F = (V/nt)\ln(C_0/C_t)$ , where C<sub>0</sub> and C<sub>t</sub> are the algal concentrations at time 0 and time t respectively and n = number of mussels. About one hour before an experiment a low concentration algal cells (2–4 × 10<sup>3</sup> cells ml<sup>-1</sup> which do not saturate the digestive system, see later) were added to the aquarium to stimulate the mussels to open fully (maximal filtration rate).

**Conversion factors:** The following conversion factors were used to estimate growth of *Mytilus edulis* in order to calculate the minimum algal concentration required for maximal growth as observed in nature. Algal cells, *Rhodomonas baltica*: 1 mg C = 11.4 cal (Platt and Irwin 1973) = 47.72 J; carbon content = 36.7 pg C cell<sup>-1</sup> (Kiørboe et al. 1985) equivalent to 47.72 × 10<sup>-9</sup> × 36.7 = 1.75 µJ

cell<sup>-1</sup>. Algal cells, *Phaeodactylum tricorutum*: 8.5 pg C cell<sup>-1</sup> (assuming 50% carbon content) (Riisgård and Randløv 1981). Mussels, *M. edulis*: Filtration rate (F, l h<sup>-1</sup>) as a function of size (W, g dry wt of soft parts):  $F = 7.45W^{0.66}$  (Møhlenberg and Riisgård 1979). Maintenance respiration rate R<sub>m</sub>(µl O<sub>2</sub> h<sup>-1</sup>) as a function of size (W, g dry wt) in mussels:  $R_m = 475W^{0.663}$  (Hamburger et al. 1983); 1 ml O<sub>2</sub> h<sup>-1</sup> = 4.75 cal h<sup>-1</sup> = 19.88 J h<sup>-1</sup> = 5522 µJ s<sup>-1</sup> = 5522 µW. Maximal growth (G<sub>max</sub>) (see Table 8 in Jørgensen 1990) in juvenile *M. edulis* was obtained in mussels transferred in net bags to the eutrophicated Limfjord, Denmark, the mean growth of soft parts being 7.0 ± 1.7 mg dry wt d<sup>-1</sup> for mussels transferred to Station 1, 2, 4, 5 and 6 in areas without oxygen depletion in the bottom waters (Riisgård and Poulsen 1981); 1 mg dry wt of soft parts = 4.9 cal (Dare and Edwards 1975) = 20.51 J.

#### RESULTS

##### Filtration Rate Experiments

The filtration rate was measured at 15 and 9°C at different algal concentrations. Reduction in the concentration of algal cells due to grazing of the mussels was fast and constant (i.e., the clearance, expressed by the slope of the line fitted for the algal reduction in a semi-logarithmic plot, was high and appeared constant) at the algal concentration range between about 2–6 × 10<sup>3</sup> cells ml<sup>-1</sup> (Fig. 1A & B). The mean filtration rates at 15 and 9°C were 40 ± 1.5 and 31 ± 3.4 ml min<sup>-1</sup>, respectively (Fig. 1A). In experiments where the algal concentration was first kept between about 2–4 × 10<sup>3</sup> cells ml<sup>-1</sup> for at least one hour and then more algal cells added to give concentrations above 10<sup>4</sup> cells ml<sup>-1</sup> the filtration rate gradually decreased (Fig. 1C–E & Fig. 2). The decrease in filtration rate was observed to be correlated with a reduced valve gape, withdrawn mantle edges, a narrowing of the exhalant siphon, and production of pseudofeces. In experiments at low algal concentrations all mussels were always fully open, and the maximal filtration rate was constant during the period of 28 days at 9°C (Fig. 1, open circles).

The time during which the filtration rate remained maximal after the algal concentration was raised from about 3 × 10<sup>3</sup> cells ml<sup>-1</sup> to 1.5, 3 and 4 × 10<sup>4</sup> cells ml<sup>-1</sup> is shown in Table 1. The time during which the filtration rate capacity was maintained decreased with increased algal concentration. The total number of cells ingested during the period of maximum filtration rate was about constant, i.e. 43 ± 2.6 × 10<sup>6</sup> cells mussel<sup>-1</sup> (Table 1). The mean algal cell volume was about 110 µm<sup>3</sup> and the total volume of algae ingested was thus about 4.7 mm<sup>3</sup>.

The filtration rate as a function of time, after an initial period of maximal filtration rate at high algal concentrations, decreased as shown in Figure 3. The pattern seemed not to be influenced by the algal concentration, indicating

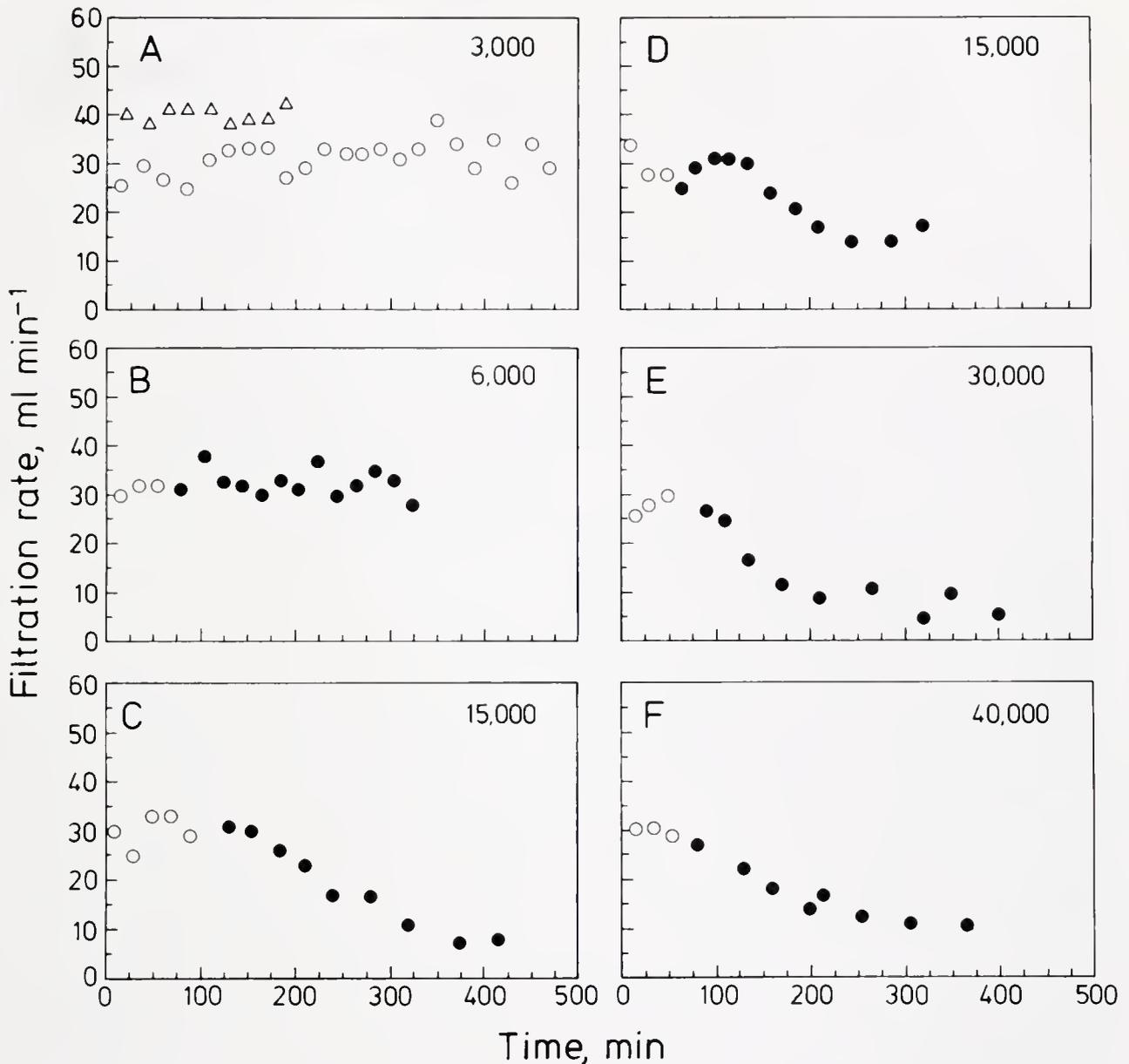


Figure 1. *Mytilus edulis*. Mean filtration rate in a group of 25 mussels as a function of time at different algal (*Rhodomonas baltica*) concentrations and after different acclimatization time at 9°C except in one case (A) where the filtration rate was measured at 15°C previous to the 9°C acclimatization period. Open symbols refer in all cases to measurements performed at  $3 \pm 1.2 \times 10^3$  cells ml<sup>-1</sup>. (A) Filtration rate measured at  $3 \pm 1.2 \times 10^3$  cells ml<sup>-1</sup> after 28 days at 15°C (triangles) and after 10 days at 9°C (=Day 10) (circles). (B) Filtration rate measured on Day 11; filled symbols:  $6 \pm 2 \times 10^6$  cells ml<sup>-1</sup>. (C) Day 14;  $15 \pm 5 \times 10^3$  cells ml<sup>-1</sup>. (D) Day 15;  $15 \pm 5 \times 10^3$  cells ml<sup>-1</sup>. (E) Day 17;  $3 \pm 1 \times 10^4$  cells ml<sup>-1</sup>. (F) Day 21;  $4 \pm 1 \times 10^4$  cells ml<sup>-1</sup>.

that the filtration rate was not being adjusted to the ambient algal concentration. After the gut capacity (about 4–5 mm<sup>3</sup> algae) was exceeded the mussels apparently responded in a consistent manner by reducing the degree of valve gaping.

#### Estimation of Growth

The maximal growth of a "standard" 173 mg dry wt (23 mm shell length) *Mytilus edulis* under optimal food conditions was 7 mg dry wt d<sup>-1</sup> (in the Limfjord) corresponding

to  $G_{\max} = 1662 \mu\text{W}$ . The maintenance metabolism ( $R_m$ ) as expressed by the rate of oxygen consumption is estimated at 148.4  $\mu\text{l O}_2 \text{ h}^{-1}$  corresponding to 817  $\mu\text{W}$ . If an assimilation efficiency (AE) of 80% is assumed (Riisgård and Randløv 1981) the concentration of algal cells needed to cover the energy expenditure for maintenance and growth can be estimated. The assimilated food energy is:  $A = F$  (filtration rate = 39 ml min<sup>-1</sup>)  $\times C$  (algal concentration, cells ml<sup>-1</sup>)  $\times$  AE (0.8). When the energy cost of growth

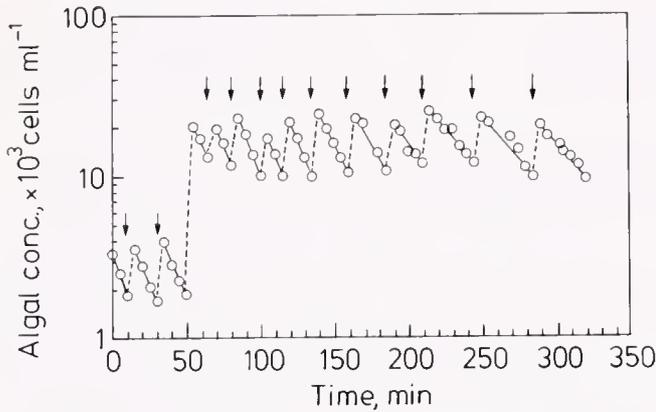


Figure 2. *Mytilus edulis*. Reduction of algal cells due to grazing by 25 mussels in an aerated aquarium. Arrows indicate additions of algal suspension. The slope of lines express the mussels' filtration rate which is reduced after some time at the high algal concentration. The calculated filtration rates are shown in Figure 1D.

( $R_g$ ) is included the energy budget expressing the amount of food energy transformed into growth is found as:  $G = A - (R_m + R_g)$ . The energy cost of growth (mainly biosynthesis) has recently been measured to be about 20% of the absorbed ration by Bayne et al. (1989) in *M. edulis*, i.e.  $R_g = 0.2G$ . The growth rate may now be expressed as:  $G = A - R_m - 0.2G$  or  $G = (A - R_m)/1.2$ . The estimated actual growth rate ( $G$ ,  $\mu W$ ) as a function of algal concentration ( $C$ , cells  $ml^{-1}$ ) may then be expressed by the equation:  $G = [(F \times AE \times C) - R_m]/1.2 = (39 \times 0.8 \times 1.75 \times C/1.2 \times 60) - (817/1.2) = 0.758C - 681 = bC - a$ . The concentration of *Rhodomonas baltica* cells needed to cover the energy cost of maintenance is found to be  $681/0.758 = 898$  cells  $ml^{-1}$ , and the concentration of algal cells necessary for maximal growth is found as:  $C = (G_{max} + a)/b = (1662 + 681)/0.758 = 3 \times 10^3$  cells  $ml^{-1}$  (or  $1.4 \times 10^4$  *Phaeodactylum tricornutum* cells  $ml^{-1}$ ). Thus, the algal concentration needed for maximal growth (as suggested to be maximum in the Limfjord, see Table 8 in Jørgensen 1990) is about three times the maintenance concentration.

TABLE 1.

*Mytilus edulis*. Maximum filtration rate ( $F$ ), total ingestion ( $I_{tot}$ ), and duration of feeding ( $t$ ) at maximum rate after algal (*Rhodomonas baltica*) concentration was elevated from  $2-4 \times 10^3$  cells  $ml^{-1}$  to the given concentration ( $C$ ) that leads to a reduced filtration rate after a certain time (cf. Fig. 1 C-D & Fig. 2).

Algal Conc. (mean) C (cells $ml^{-1}$ )	Filtration Rate (mean) F ( $ml \ min^{-1}$ )	Time of Max F t (min)	Ingestion $I_{tot}$ ( $\times 10^6$ cells)
15,000	29.0	90	39.2
15,000	28.3	105	44.6
30,000	26.0	55	42.9
40,000	27.3	40	43.7

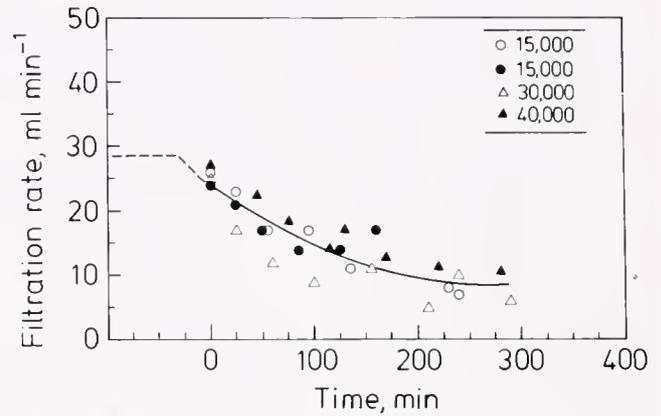


Figure 3. *Mytilus edulis*. Filtration rate as a function of time in clearance experiments performed at different algal concentrations leading to reduced filtration rate after a period during which the mussels exploit their filtration capacity (see Fig. 1D-F). Only data obtained after the previous period of maximal filtration rate are shown. The maximal filtration rate represented by the plateau and the second order regression line for all the data in the graph are shown.

## DISCUSSION

### Filtration Rate and Growth

Undisturbed *Mytilus edulis* fed up to about  $10^4$  algal (*Rhodomonas baltica*) cells  $ml^{-1}$  are typically fully open with expanding, diverging mantle edges and continuous water pumping (Fig. 1A & B). Filtration rate measurements made on mussels in this state represent the water pumping capacity and are, presumably, representative for optimal conditions in nature. If the mussels are disturbed, or if the algal concentration is elevated up to about  $1.5 \times 10^4$  cells  $ml^{-1}$  or higher, they respond by reducing the gaping of the valves. A secondary result of reduced valve gape is a reduction in width of the gill interfilament canals leading to a decline in the filtration rate (Jørgensen et al. 1988). Ill-defined relations between valve gape and filtration rate were interpreted by Jørgensen et al. (1988) to indicate a loose coupling between the width of the interfilament canals and the muscular activity that controls valve gape and extension of mantle edges. Reduced pumping rate with decreasing valve gape was found not to be a mechanism in controlling filtration, but rather a secondary effect of the mussels' response to suboptimal conditions. In the present work the pattern in reduction of filtration rate at different high algal concentrations is very similar, with no regulation of filtration rate to ambient algal concentration, ensuring a constant ingestion rate (Fig. 3). The reduction in filtration rate as a result of reduced valve gape may be interpreted as a reaction to satiation of the digestive system (Table 1), or less specifically to adverse conditions. If the ambient algal concentration is above a certain level so that the gut capacity is exceeded after some time, the mussels may respond by reducing the opening of the valves in a manner similar to suboptimal conditions or disturbance.

The reduction in filtration rate above a certain high algal concentration has been interpreted in terms of energy optimization in *Mytilus edulis* (Winter 1973), *M. chilensis* (Navarro and Winter 1982), and in the freshwater mussel *Dreissena polymorpha* (Sprung and Rose 1988). In *M. chilensis* Navarro and Winter (1982) measured the filtration rate at  $1.5$ ,  $2.5$  and  $4 \times 10^4$  *Dunaliella marina* cells  $\text{ml}^{-1}$  and found filtration rates of  $1.55$ ,  $1.05$  and  $0.79$   $\text{l h}^{-1}$ , respectively, for a  $1$  g dry-tissue wt mussel. This apparent functional response is correlated with filtration rates that are low compared to  $7.45$   $\text{l h}^{-1}$  for a  $1$  g dry tissue wt *M. edulis* clearing low algal concentrations (Møhlenberg and Riisgård 1979). Because *D. marina* and *Rhodomonas baltica* are of similar size, and because the filtration rates measured at  $3 \times 10^3$  cells  $\text{ml}^{-1}$  in the present work are in good agreement with previous measurements in *M. edulis* (Riisgård and Møhlenberg 1979, Møhlenberg and Riisgård 1979, Famme et al. 1986, Jørgensen et al. 1986a) the filtration rates measured by Navarro & Winter (1982) seems to represent values applying to adversely affected mussels at high algal concentrations. The algal concentrations of  $2$  and  $4 \times 10^4$  *D. marina* cells  $\text{ml}^{-1}$  used by Winter (1973), and  $2$  to  $9 \times 10^4$  *Chlamydomonas reinhardtii* cells  $\text{ml}^{-1}$  used by Sprung and Rose (1988) may have led to a similar overloading.

Riisgård and Randløv (1981) found that *Mytilus edulis* fed algal cells (*Phaeodactylum tricorutum*) at  $1.6 \times 10^3$  and  $2.6 \times 10^4$  cells  $\text{ml}^{-1}$  had reduced filtration rates as compared to mussels fed  $3 \times 10^3$  cells  $\text{ml}^{-1}$ . At the latter algal concentration the mussels opened their valves maximally and extended the mantle edges in contrast to mussels kept at both the lower and the higher algal concentration. Mussels fed  $2.6 \times 10^4$  cells  $\text{ml}^{-1}$  were more or less closed or had reduced the valve gape, and in the light of the present work it may be concluded that the mussels were overloaded. The mean growth rate (Group B<sub>II</sub>) was  $0.11$  mm shell length  $\text{d}^{-1}$  (or  $1.8$  mg dry tissue wt  $\text{d}^{-1}$ ) which may be compared to the maximal growth of  $0.23$  mm  $\text{d}^{-1}$  ( $7$  mg dry wt  $\text{d}^{-1}$ ) in mussels of about similar size transferred to the Limfjord (Riisgård and Poulsen 1981). The suboptimal growth in the laboratory experiment may be due to reduced metabolism (and biosynthesis) which is a secondary effect of valve closure (Jørgensen et al. 1986b). Thus, though the algal concentration was more than sufficient to ensure maximal growth the laboratory mussels may have been under metabolical constraints preventing them from exploiting their potential for growth. Such an interpretation is consistent with the absence of regulatory mechanisms for controlling the filtration rate and optimizing ingestion rate and growth (Jørgensen 1990). At an algal concentration of about  $1.4 \times 10^3$  *P. tricorutum* cells  $\text{ml}^{-1}$  ( $0.24$  mg dry wt  $\text{l}^{-1}$ ) Kiørboe et al. (1981) obtained a growth rate of  $0.24$  mm  $\text{d}^{-1}$  in *M. edulis*. This rate is similar to the maximal growth of mussels in nature, but at a higher algal concentration of about  $2 \times 10^4$  cells  $\text{ml}^{-1}$

( $0.33$  mg dry wt  $\text{l}^{-1}$ ) the growth rate was lower ( $0.19$  mm  $\text{d}^{-1}$ ). Because silt was added in the first case, but not in the latter, Kiørboe et al. concluded that *M. edulis* depends on suspended bottom material to fully exploit its filtration capacity and to reach growth rates similar to peak rates in nature. An alternative explanation of the faster growth at the lower algal concentration is that the mussels may have been adversely affected at the highest concentration, whereas the algal concentration in the silt experiment was near to the concentration needed for maximal growth in mussels exploiting their filtration rate capacity as calculated in the present work. The calculations based on maximally growing mussels show that the growth potential may be utilized at an algal (*Rhodomonas baltica*) concentration of about  $3 \times 10^3$  cells  $\text{ml}^{-1}$ , and it seems a though mussels may be adapted to exploit their filtration capacities only at low algal concentrations. Very low algal concentrations lead to shell closure and reduced filtration rate (Riisgård and Randløv 1981) and metabolism (Jørgensen et al. 1986b). If the above statements (which do not include possible role of silt or seston quality) are correct, the generally low growth rates obtained in laboratory studies on a pure algal diet, as compared to maximal growth rates in nature (cf. Table 6 in Kiørboe et al. 1981), may be due to the use of unnaturally high algal concentrations which lead to sub-optimal conditions (valve closure, reduced metabolism and reduced biosyntheses/growth). This conclusion emphasizes the need for more growth experiments at low algal concentration in the laboratory. But more knowledge about actual algal concentrations in the immediate vicinity of benthic suspension-feeding bivalves is also needed. The food particle concentration in the boundary-layer over a mussel bed may be extremely low and limit growth (Fréchette and Bourget 1985a, b, Fréchette et al. 1989). There is increasing evidence that growth of benthic suspension-feeding bivalves may be food-limited because the phytoplankton concentrations above dense populations of mussels may be more or less depleted. Measurements of vertical gradients in algal concentration over a mussel bed in the St. Lawrence River estuary showed a significant reduction in algal concentration close to the bed (Fréchette et al. 1989). Another case may serve as an example. According to Jørgensen (1980) the average population density of *M. edulis* in a mussel bed in the Limfjord, Denmark, was  $1550$  ind  $\text{m}^{-2}$  with an average shell length of  $5$  cm (above  $0.5$  g dry tissue wt). The amount of water filtered by the mussels is calculated to be about  $180$   $\text{m}^3$   $\text{m}^{-2}$   $\text{d}^{-1}$ , i.e. equivalent to about  $20$  times the whole water column per day. The amount of algae needed to cover the mussels' energy expense for maintenance is calculated to be  $25$  g C  $\text{d}^{-1}$ . The phytoplankton productivity is about  $0.6$  g C  $\text{m}^{-2}$   $\text{d}^{-1}$  and from this it is clear that the mussels are highly dependent on the supply of algae transported to the mussel bed with water currents. If the vertical mixing is of minor importance and the water available to the mussels is re-

stricted to 0.5 m above the bed the mussels must recirculate the same bottom water many times a day, and the mean algal concentration may therefore be very low. To nourish from such extremely meagre waters mussels seem to have developed filter-, pump- and digestive systems that are able to cope with naturally low algal concentrations. Rather, they are adapted to survive conditions not compatible with the full exploitation of the growth potential, cf. beneficial effects of transferring mussels from the natural beds to e.g. net bags (see Table 13 in Jørgensen 1990).

A functional response similar to that seen in Fig. 3 has previously been found in early veliconchia of *Mytilus edulis* (Riisgård et al. 1980) and veliger larvae of the hard clam *Mercenaria mercenaria* (Riisgård 1988) fed *Isochrysis galbana* cells. At low algal concentrations ( $2 \times 10^3$  to  $4 \times 10^3$  cells  $\text{ml}^{-1}$ ) the clearance of *M. edulis* larvae was high and constant during 8 hours. At high algal concentrations (above  $2 \times 10^4$ ) the clearance capacity was only maintained until the stomach was full. The time at which this took place was indicated by a shift in the relationship between clearance and algal concentration in a semi-logarithmic plot. Similar shifts in slope of feeding curves were seen in the present work (Fig. 2). Experiments with the suspension-feeding polychaete *Sabella penicillus* exposed to algal concentrations above  $10^4$  *Rhodomonas* cells  $\text{ml}^{-1}$  have also shown such shifts; but the clearance was high and constant (for at least 8 h) at algal concentrations below about  $4 \times 10^3$  cells  $\text{ml}^{-1}$ . At higher concentrations it appeared that the gut capacity was exceeded thus

leading to a reduced filtration rate (Riisgård and Ivarsson 1990). Thus, a decrease in filtration rate in marine suspension-feeders as a response to high algal concentration may simply be due to an overloading of the feeding system. This stresses the importance of performing laboratory experiments at naturally low algal concentrations as well as including non-algal suspended matter, such as silt, in order to interpret laboratory findings in a meaningful ecophysiological context.

#### Effects of Temperature

The effects of temperature on the mussel pump have recently been thoroughly studied by Jørgensen et al. (1990). The filtration rate ( $F$ ,  $\text{ml min}^{-1}$ ) as a function of temperature ( $t$ ) in the same group of 25 mussels used in the present study could be expressed as:  $F = 22.8 + 1.13t$  ( $6-16^\circ\text{C}$ ;  $r = 0.67$ ). In the present work the filtration rates at 15 and  $9^\circ\text{C}$  were found to be  $40 \pm 1.5$  and  $31 \pm 3.4$   $\text{ml min}^{-1}$ , respectively, which may be compared to 39.8 and 33.0  $\text{ml min}^{-1}$  as estimated from the above equation. Thus, the filtration rate in cold adapted mussels was unchanged during a two months acclimatization period to higher temperatures. This confirms the previous finding of the absence of temperature adaptation in *Mytilus edulis* (Jørgensen et al. 1990).

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## PRODISSOCONCH SHELL CHARACTERISTICS AS INDICATORS OF LARVAL GROWTH AND VIABILITY IN *PECTEN MAXIMUS* (LINNAEUS, 1758)

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**ABSTRACT** Differences in viability were observed in cultures of *Pecten maximus* larvae released from broodstock collected in the Bay of Saint-Brieuc (France). Spawnings which were not synchronous with the spawning event in the field produce more anomalous D-shaped larvae, a decrease in growth and survival, and, finally a poor metamorphosis rate. A negative relationship between the shell growth and the curvature of the two valves of the prodissoconch was revealed; thus, an index called  $Dim_{90}$  was established. It corresponds to the depth at a height of 90  $\mu\text{m}$ . The  $Dim_{90}$  is an estimation of the growth potential of an early stage larva. It appeared that maintaining adults at a low temperature ( $14 \pm 1^\circ\text{C}$ ) before spawning for more than one month induced a high proportion of larvae characterized by a high  $Dim_{90}$  ( $>56 \mu\text{m}$ ) and low growth, with resulting death before metamorphosis. It is likely that the variability in the larval competence expected in the early larval stage by the knowledge of the  $Dim_{90}$  has an endogeneous origin. In our experiments, it may have resulted from the poor quality of the ovocytes released, due to inadequate thermal conditions during gametogenesis.

**KEY WORDS:** scallops, *Pecten maximus*, prodissoconch, larval growth, viability

### INTRODUCTION

The viability of marine bivalve larvae reared in a hatchery is partially determined by environmental conditions during the sexual maturation of spawners. Thus, for *Ostrea edulis* and *Mytilus edulis* populations, temperature and nutritive stresses imposed on adults throughout gametogenesis result in an apparent loss of quality of the gametes (Helm et al. 1973, Bayne 1972, Bayne et al. 1975). This is followed by an increase of abnormalities of the prodissoconch I and a decrease in viability and larval growth rates. Such variations in larval competence have been proposed to explain some irregularities in the recruitment of scallops (*Pecten maximus*) in the Bay of St Brieuc, (France) (Boucher 1989).

Experiments were conducted in a hatchery during the natural spawning cycle of *Pecten maximus* on adults regularly collected from the Bay of St-Brieuc (Brittany, France) to determine the influence of environmental conditions during sexual maturation on growth performance of the resulting larvae. Adult scallops were also held at cold temperature to assess this temperature impact on larval viability. The differences of the various cultures were compared and related to spawning events in the wild population.

In our studies, the larval viability is based not only on survival, growth, abnormalities of the prodissoconch shape and rate of metamorphosis, but also on a recently defined index, the  $Dim_{90}$ , which corresponds to the depth of shell (maximum intervals distance) at a height of 90  $\mu\text{m}$  (Salaun et al. 1989). This individual index, which is an expression of the shell convexity, can be used as a potential

growth index and can be determined at any time during the larval period.

Concurrent studies on dynamic of the ovocyte production (Paulet et al. 1989) and oocyte viability (Dorange et al. 1989) were conducted on the same populations as part of a study on the 'Programme National sur le Déterminisme du Recrutement (PNDR)' of the scallop *Pecten maximus* (Boucher 1985). Today there is a need for a real understanding of these processes to provide a better basis for stock management and extensive shellfish mariculture (Boucher and Dao 1990).

### MATERIAL AND METHODS

#### 1. Origin of Spawners, Conditioning and Induced Spawning Date

Sampling of mature adult *Pecten maximus* in the Bay of St-Brieuc (France) was carried out weekly from late June until September 1987. Individuals were transferred to a hatchery at Argenton (France) and maintained into recirculating seawater tanks at  $18^\circ\text{C}$ . One or two days after collection, spawning of 20 ripe individuals was attempted using thermal stimulation. There, the induced spawning dates were correlated with the wild spawnings defined later on the basis of the gonado-somatic index variations (Paulet 1988) and planktonic larval abundance.

Another broodstock of ripe *P. maximus* collected in early July was held at  $14^\circ\text{C}$ , i.e.,  $2^\circ\text{C}$  less than the minimum required for a natural release of gametes in the Bay of St-Brieuc (Boucher 1985). Three batches were conditioned and spawnings induced after a delay of 9, 16 and 35 days.

## 2. Obtaining and Rearing Larvae

When male and female gametes were gathered separately in beaker filled with 1.5 l of filtered seawater. Water was frequently changed to avoid self-fertilization (Comely 1972). The oocytes were fertilized with sperm from separate individuals (Gruffydd and Beaumont 1972). The eggs from 3 to 6 spawners were then separately stored at a density of  $50 \text{ eggs/ml}^{-1}$  in conical tanks containing 150 l of  $1 \mu\text{m}$  filtered seawater. At  $17 \pm 1^\circ\text{C}$ , straight-hinged veligers with newly developed prodissoconch I appeared two days after fertilization. A subsample of D-shaped larvae from each batch was pooled together giving a density of approximately  $5 \times 10^6$  veligers per tank of 450 l. Cultures were then maintained following the protocol described by Buestel et al. (1982).

The seawater in the culture containers was changed three days a week. Loss of slow growing larvae was prevented by filtering on a nylon sieve of  $45 \mu\text{m}$  mesh size. To limit bacterial growth (Walne 1958, Comely 1972),  $8 \text{ mg/l}^{-1}$  chloramphenicol were added to each culture (Le Pennec and Prieur 1977). At the beginning of the veliger stage, two days after fertilization, larvae were fed daily on a mixed algal diet of two phytoflagellates *Pavlova lutheri* and *Isochrysis galbana* (T. iso clone) ( $40 \text{ cells} \cdot \mu\text{l}^{-1}$ ), and an algal diatom *Thalassiosira pseudonana* ( $10 \text{ cells} \cdot \mu\text{l}^{-1}$ ). Large larvae reaching metamorphosis as indicated by the formation of a double-ring at the outline of the shell (Gérard et al. 1989), were gathered using a large mesh ( $150 \mu\text{m}$ ) sieve. They were then transferred for settlement into sieve trays ( $120 \mu\text{m}$  mesh size) at a density of 100,000 pediveligers per tray. Larvae discarded after the first sieving were put back into the rearing tanks until new pediveligers appeared. Fifteen days after transferring these com-

petent veligers, spat were counted and the rate of metamorphosis calculated:

$$\text{Metamorphosis rate (\%)} = \frac{\text{Number of postlarvae}}{\text{Number of D-shaped larvae}}$$

## 3. Larval Measurements and Survival Estimate

Growth and survival were measured every two days during development, by removing subsamples from the cultures in order to number live, dead and abnormal. Length of the shell (Fig. 1) was measured for 50 larvae, using a Nikon profile projector. The larval growth rate was calculated up to the point of transfer of first pediveligers into the sieve trays ( $t_1$ ):

$$\text{Growth rate} = \frac{\text{Mean shell length } (t_1) - \text{mean length of D-shaped larvae } (t_0)}{\text{Age } (t_1) - 2 \text{ days } (t_0)} \quad (\mu\text{m/day})$$

Every second days, at least 300 veliger larvae were put in centrifugal cones with 1.5 ml seawater and stored in a freezer at  $-25^\circ\text{C}$ . This type of preservation differed from that described by Stephenson and Chanley (1979), Carriker and Palmer (1979), Foighil (1986) but is sufficient for only shell observations. Furthermore, the shell remains slightly open which facilitating preparation for the ultramicroscopical examination. To clear the shell of organic matter, the larvae were immersed in a 5% solution of sodium hypochlorite (Rees 1950) and later rinsed in distilled water. Then, using a mounted fine needle under a binocular microscope ( $\times 40$ ), each larval shell was gently laid down on a 1 cm diameter stub, stripped with self-adhesive copper so that the edge of the shell was directly in contact with the stub (Fig. 1a). The samples were gold-coated and viewed

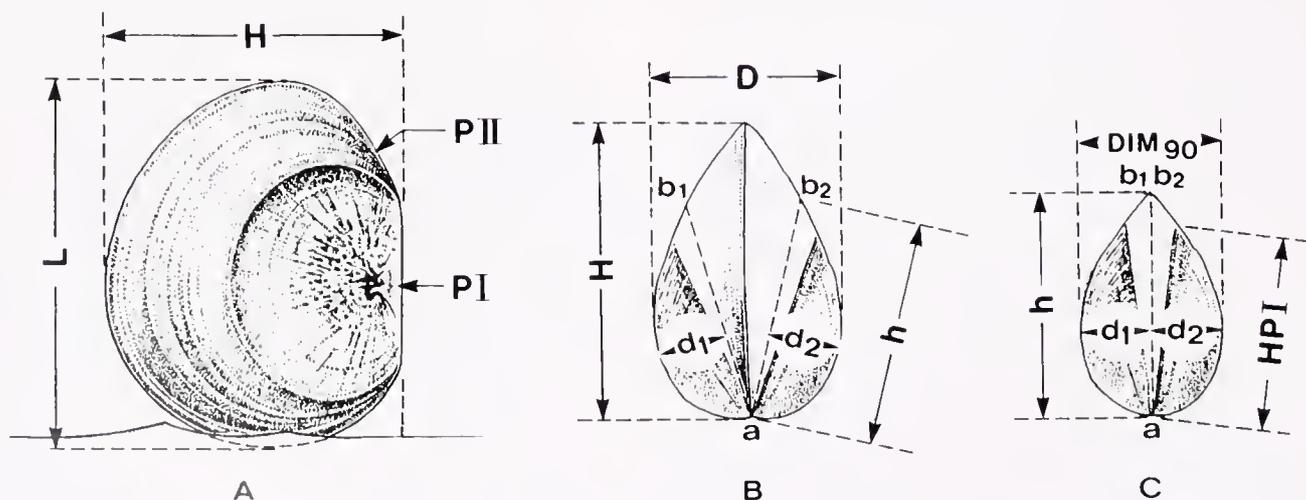


Figure 1. Measurements of the prodissoconch. a) Larval shell on the stub (view of the left valve). b) Position of the prodissoconch in order to observe the profile of the valve. c) Shell represented for a height of  $90 \mu\text{m}$ . L = length; H = height; D = depth; PI = prodissoconch I; PII = prodissoconch II; HPI = height of prodissoconch I; a = hinge axis;  $b_1$  and  $b_2 = 90 \mu\text{m}$ ;  $d_1$  and  $d_2 =$  maximum distances perpendicular to the straight lines  $ah_1$  and  $ah_2$ ;  $\text{Dim}_{90} =$  maximal interval distance for a shell height of  $90 \mu\text{m}$  (depth at  $90 \mu\text{m}$ ;  $d_1 + d_2$ ).

under a Jeol JSM-35 scanning electron microscope. Precise measurements of the prodissoconch profiles were required to orientate the hinge axis perpendicular to the stub surface (Fig. 1).

The maximum intervalve distance for a shell height of 90  $\mu\text{m}$  ( $\text{Dim}_{90}$ ) was measured on scanning electron photomicrographs taken at a magnification of exactly 600. The  $\text{Dim}_{90}$  which corresponds to the depth of an individual three or four days old can be determined at any time of its larval life. The precise method of calculation is illustrated in Figure 1.

The height of prodissoconch I (HPI—Fig. 1), characterized by a punctate texture was also calculated on photomicrographs of larval shell aged of 4 days or more. The prodissoconch I shows an abrupt change in curvature on its edge and is generally separated from the prodissoconch II by a prominent growth line.

Student's t-test was used to compare the mean heights for each culture studied. The confidence interval of the mean height or length was calculated at 0.05%. Statistical calculations were performed through the statgraphics program.

## RESULTS

### 1. Shape Anomalies of the Larval Shell

a) In any cultures of larvae whatever the origin of spawning a few veligers examined by electron microscopy scanning showed malformations of their shell (Pl. 1). Sometimes the shell surface was wavy in outline, or visible cracks cut the natural striations of the prodissoconch in a perpendicular way. In other individuals the valves were asymmetric to the point that the shell could not be fully dosed. Deformities, strongly marked on the prodissoconch II, already existed on prodissoconch I. Most of these malformations were visible with light microscopy: their influence on the rearing success will be first analyzed (2).

b) A second anomaly, revealed only by scanning microscopy, affected the curvature of the valves of the prodissoconch, quantified by the measurement of the depth at a height of 90  $\mu\text{m}$  ( $\text{Dim}_{90}$ ). High values of this index characterize abnormality of the larval shell. The effects of these two types of shape anomalies (irregularities of the shell surface + high  $\text{Dim}_{90}$  value) on growth and survival will be discussed below (3).

### 2. Impact of Delayed Spawning on Rearing Success

In groups of larvae obtained at the same time as the wild spawning event (pool 1), anomalous D-shaped larvae determined under light microscopy comprised only 4.2% of the total (Table 1). At the end of the larval life, when the first pediveligers were transferred into the trays, the survival rate was high 92.3%. Moreover, in this pool, growth and metamorphosis rates were the highest. Time-delayed spawning (pools 2, 3, 4, 5) produced an increase of anomalous

larvae from 11.1 to 17.3%. There was, however, no significant trend in the characteristics of the different rearings related to the duration of the time-lag. These delayed spawnings all resulted in a slow-down in the larval growth rate, decrease in the survival rate and, finally, decrease in the metamorphosis rate (Table 1). Rearing efficiency was poor when spawning was induced after maintaining mature adults at 14°C. The imposed delay of the spawning resulted in an increase in mortality at the end of rearing, where only the largest individuals remained alive. In this case, the metamorphosis rate was zero nearly or zero.

### 3. Relationship Between the Prodissoconch Abnormalities, Growth and Survival

Comparison of mean growth and survival estimates of individuals from two different batches (imposed delayed spawnings (pool 8) and induced spawnings in phase with the natural cycle (pool 1)) was used to establish the relationship between abnormalities of the larval shells by scanning microscopy (contour deformities or high convexity:  $\text{Dim}_{90} > 56 \mu\text{m}$ —see point 1) revealed and individual viability (Fig. 2). Very fine shell observations can be obtained by scanning microscopy, so more anomalies as a high  $\text{Dim}_{90}$  valve can be detected.

In pool 1, the proportion of larvae characterized by irregular prodissoconch or  $\text{Dim}_{90} > 56 \mu\text{m}$  varied from 31 to 36% throughout the entire experiment. The growth was linear and fast from day 1 to 16; reduction in growth rate at a length of 185  $\mu\text{m}$  occurs as the pediveliger stage is reached. Survival up to 18 days was high (>90%).

Larvae of spawners subjected to thermal stress for 35 days had a shell anomaly rate of 60–73% up to day 14. A decrease in this proportion to 16% between day 16 and 23 simultaneously a sudden increase of mean length is explained by the high mortality of these anomalous larvae occurred after day 18. These incompetent larvae was responsible for the decreasing growth rate from day 4 to 18. Consequently, the difference in growth rate observed between the two batches resulted in a different proportion of slow growing larvae characterized by an abnormal high shell convexity.

### 4. Growth Rate and Prodissoconch Curvature ( $\text{Dim}_{90}$ ) Relationship

When the larval shell height reached 90  $\mu\text{m}$ , i.e., one or two days after the appearance of veligers in the rearing tanks, the maximal intervalve distance ( $\text{Dim}_{90}$ ) ranged from 45  $\mu\text{m}$  to 75  $\mu\text{m}$  (Fig. 3). A dispersion diagram at day 7 and 14 in pools 1 and 8 gives an idea of the  $\text{Dim}_{90}$  and larval relationship. The proportion of individuals with a high  $\text{Dim}_{90}$  decrease with increasing height. This phenomenon was more apparent for veligers showing hard deformities of their shell (not represented on the graph), particularly numerous in the pool 8. For such individuals, the

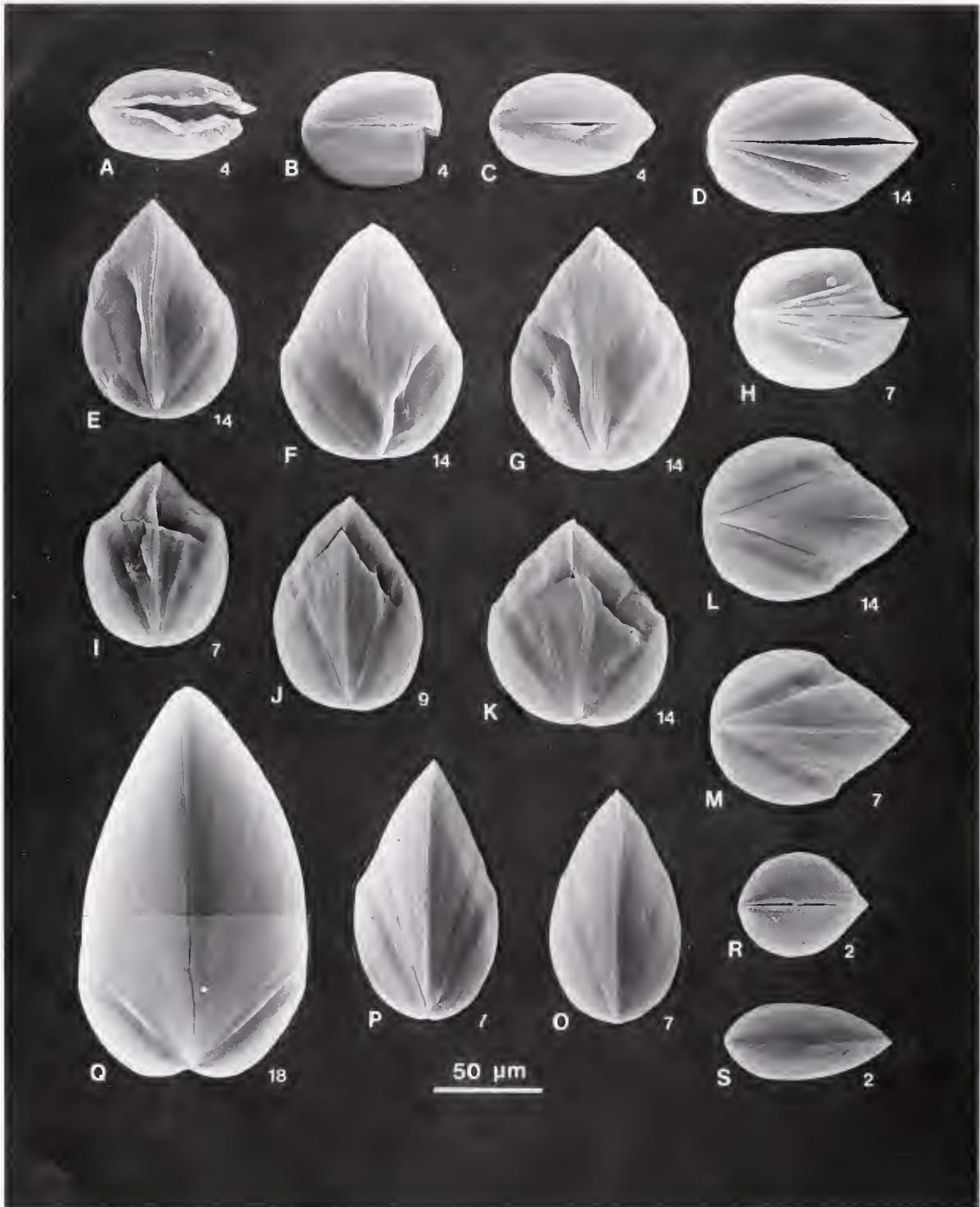


Plate I. Differences of curvature between normal and anomalous larval shells. Prodossoconchs with relief deformities (photos A to K): —shells which can not be fully closed (A to D), —shell surface wavy in outline (E to H), —cracks cutting the natural striation of the prodossoconch (I to K), Prodossoconchs showing a soft relief (photos L to S): —abnormal shell characterized by a high  $Dim_{00}$  (L and M), —normal shell characterized by a low  $Dim_{00}$  (O to Q), —the micrographs R and S represent D-shaped larvae released from genitors from the Bay of Brest. See differences in height and depth between the two prodossoconch I. NB: The age of larvae is indicated under each photomicrographs.

TABLE 1.

Rearing efficiency of larval cultures spawned at different times relative to the natural spawning event characterized by larval abnormalities, growth, survival and metamorphosis rates.

Date of Induced Spawns and Time Difference (days) between Spawns in the Bay of St Brieuc	Pool N°	Anomalous D-Shaped Day 2 (%)	Larval Growth Rate ( $\mu\text{m}/\text{day}$ )	Survival at the End of Larval Life (%)	Larval Life Duration (days) Pediveliger Stage	Metamorphosis Rate (%)	
Simultaneous :	$t_2$	1	4.2	4.91	92.3	18	2.4
Untimely	$t_1 - 7$	2	17.3	3.33	50	18	1.1
or	$t_1 + 3$	3	16.4	3.87	53.9	18	1.0
delayed	$t_1 + 9$	4	13.2	4	48.8	18	0.1
spawnings	$t_3 + 5$	5	11.1	2.73	2	27	0.3
Imposed delayed	$t_1 + 9$	6	6.4	3.26	24.1	21	0
of spawning by	$t_1 + 16$	7	9.5	3.64	27.3	19	0.2
maintaining adults at 14°C	$t_1 + 35$	8	12.2	3.24	12	23	0

Spawns observed in Saint-Brieuc Bay:  $t_1$ : July 6,  $t_2$ : July 23–29,  $t_3$ : Aug 5–13. Anomalous larvae at two days: larval shells with a deformed D-shape or veligers with an unretractile velum (observations under light microscopy).

$\text{Dim}_{90}$  approaches 85  $\mu\text{m}$ , giving them a globular shape (see Plate I).

For a better characterization of this relationship, individuals were classified into four groups of arbitrarily defined  $\text{Dim}_{90}$ :  $\text{Dim}_{90} \leq 52 \mu\text{m}$ ;  $52 \mu\text{m} < \text{Dim}_{90} \leq 56 \mu\text{m}$ ;  $56 \mu\text{m} < \text{Dim}_{90} \leq 60 \mu\text{m}$  and  $\text{Dim}_{90} > 60 \mu\text{m}$ . The mean height of individuals for each  $\text{Dim}_{90}$  class experiments 1 and 8 (Fig. 4) was significantly ( $p < 0.05$ ) and negatively related to the value of the  $\text{Dim}_{90}$  (Table 2): two days after fertilization. Individuals with low values of  $\text{Dim}_{90}$  tended to have a larger prodissococonch I. Larvae characterized by large prodissococonch I tended to be thin when they reached 90  $\mu\text{m}$ ; whereas small D-shaped larvae tended to be globular in shape at 90  $\mu\text{m}$ . In pool 1, differences between the mean height of prodissococonch II were kept up to the 7th day ( $p < 0.01$ ); thin individuals with  $\text{Dim}_{90} \leq 52 \mu\text{m}$  were still the highest. In pool 8, the mean heights were more concentrated and there was only the  $\text{Dim}_{90}$ -class  $> 60 \mu\text{m}$  which differ with the others ( $p < 0.01$ ). When comparing the height of  $\text{Dim}_{90}$  classes of larvae within pools 1 and 8 for day 7, there were no significant differences except for the  $\text{Dim}_{90}$ -class  $< 52 \mu\text{m}$  ( $p < 0.05$ ). After the 7th day a slow-down of the growth was observed in both pools. At day 14, difference between means height in the three  $\text{Dim}_{90}$ -classes  $\leq 60 \mu\text{m}$  were highly significant in both pools 1 and 8 ( $p < 0.001$ ), whereas means height of individuals with the highest  $\text{Dim}_{90}$  ( $> 60 \mu\text{m}$ ) did not differ significantly. Thus differences in growth rate occurred between larvae, related to their  $\text{Dim}_{90}$  and origin. No significant differences were detected between the two- $\text{Dim}_{90}$ -classes  $< 56 \mu\text{m}$ . In pool 8, which originated from the delayed spawning, the individuals characterized by a  $\text{Dim}_{90} < 56 \mu\text{m}$  reached the 160  $\mu\text{m}$  height at day 20, i.e., 6 days after those released simultaneously to a spawning observed in the field (pool 1). Individuals from the largest  $\text{Dim}_{90}$ -

class ( $> 60 \mu\text{m}$ ) in pool 1 reached a maximum mean height of 120  $\mu\text{m}$  after 14 days of rearing and did not grow any further. No similar conclusions concerning maximum height and delay could be verified for individuals of the delayed spawning batch (pool 8) and  $\text{Dim}_{90} > 56 \mu\text{m}$ . In fact such individuals with high  $\text{Dim}_{90}$  disappeared during the mass mortality after 14 days.

Maintaining adults at reduced temperatures before spawning favored both the formation of abnormal unviable larvae with globular prodissococonchs or produced a decrease in growth for every larva whatever be its  $\text{Dim}_{90}$ .

## DISCUSSION

Of the criteria available for characterizing bivalve larvae those which have been used most commonly involve either hinge morphogenesis, general shell shape or dimensions such as shell length and height (Rees 1950, Loosanoff and Chanley 1966, Loosanoff 1966, Chanley and Andrews, 1971, Le Pennec 1978, Lutz et al. 1982, Tremblay et al. 1987). The depth is scarcely used and few authors have measured this parameter on prodissococonchs because of the difficulties of manipulating larvae under optical microscopes. Chanley and Van Engel (1969) and Stephenson and Chanley (1979) established a three dimensional graph showing the combined evolution of length, height and depth in *Montacuta percompressa* and *Chione stutchburgi*. These authors indicated that each bivalve species might be characterized by a polyhedron integrating the three dimensions which might facilitate their identification in planktonic samples.

Depth as well as length or height increases throughout and cannot therefore be used as a growth index. The depth at a fixed height of 90  $\mu\text{m}$  ( $\text{Dim}_{90}$ ) characterizes the larvae as early as one or two days after shell formation and has been previously used to allow analyze morphogenesis ab-

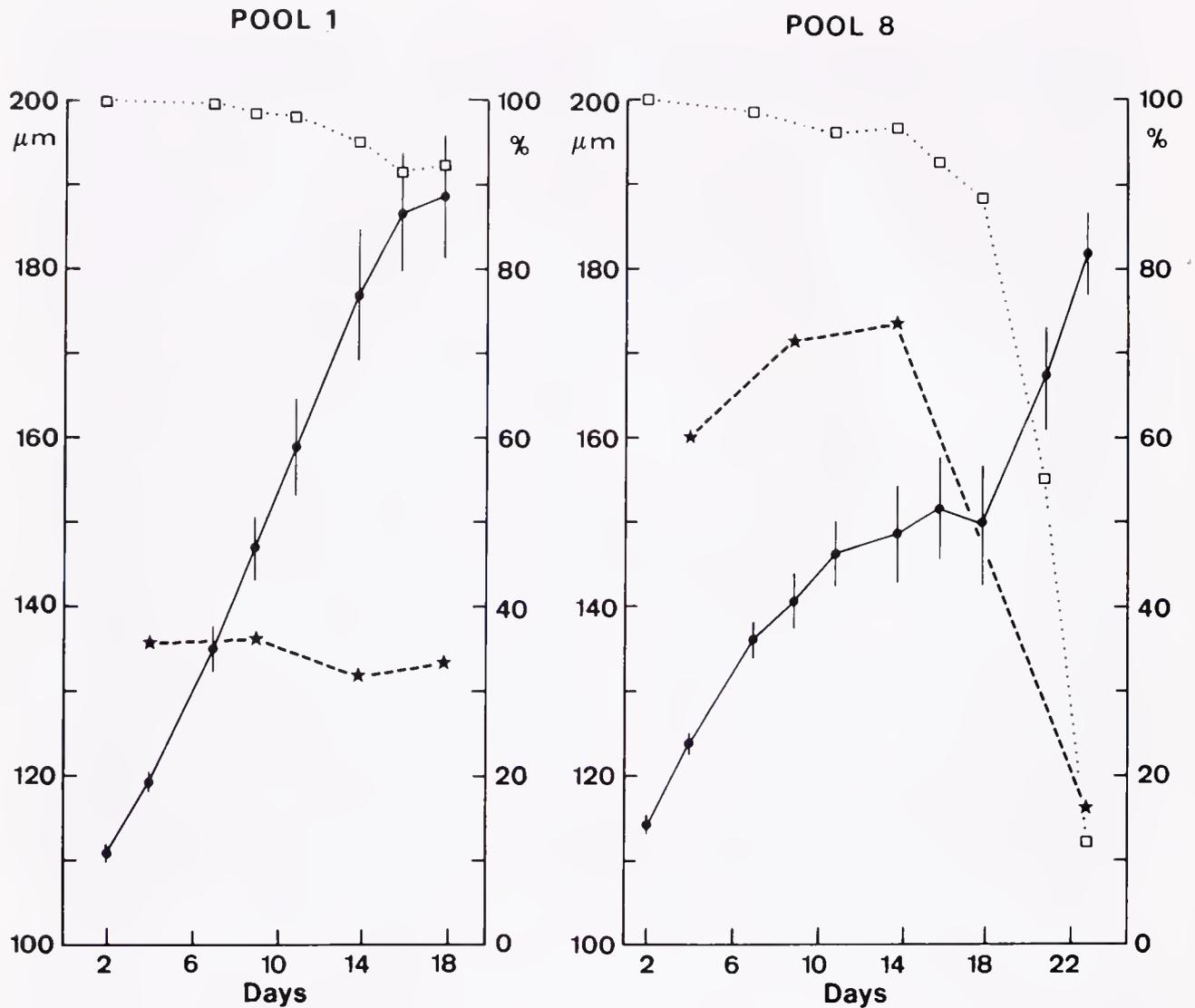


Figure 2. Larval growth (●—●), survival (□••□) and proportion of anomalous veligers characterized by abnormalities of the shell shape or high  $Dim_{90}$  ( $<56 \mu\text{m}$ ) (★--★), in pools 1 and 8.

normalities. This measure available any time during the larval life, gives an estimation of the potential growth. In larvae, a high  $Dim_{90}$  can be associated to a slow down of growth and a reduction in chances of survival. In culture the smallest straight-hinge larvae, at two days will be characterized by a high  $Dim_{90}$  and will constitute "runts of the litter." In such individuals the globular shell shape observed as early as the first larval day, may be accentuated later. Larvae are thus distinguished by a prominent umbo. This one is more or less precociously visible in conformity with the degree of the shell convexity (Salaün 1985). This variability of the larval shell curvature at a fixed height related to differences of larval growth and viability should be researched in other species of bivalve.

In bivalves the malformations of the larval shell can have an exogenous origin. For example veligers of *My-*

*tilus edulis* reared in presence of hydrocarbures exhibit abnormal shell shape (Le Pennec and Le Roux 1979). In larvae of American oyster and hard-shell clam, Calabrese (1972) showed that detergents induced high mortality and produced on survivals shape abnormalities. As already noted in *Pecten maximus* larvae reared from spawners from the Bay of Brest slow growing individuals have a convex prodissococonch 1, indicating that this anomaly has an endogenous origin (Salaün et al. 1989). The increased percentage of abnormal individuals released from delayed spawning implies that it is determined by factors acting during the production of gametes.

In *Mercenaria mercenaria*, adults maintained at  $15^{\circ}\text{C}$  for 9 weeks delayed spawning and produced abnormal larvae which fail to metamorphose (Carriker 1961). Cullinly (1974) observed that in *Placopecten magellanicus*

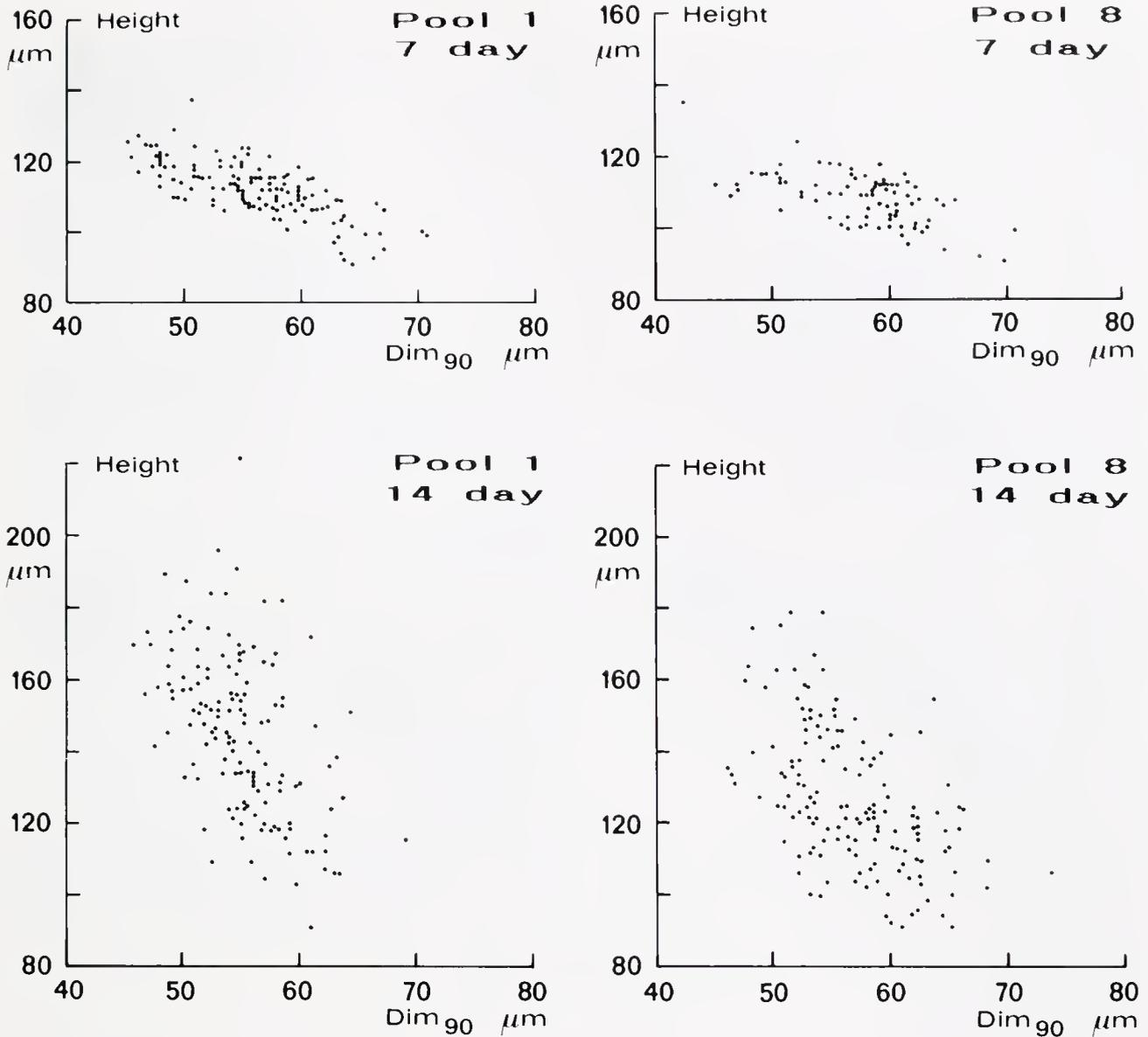


Figure 3. Height of shells versus depth at height = 90  $\mu\text{m}$  ( $\text{Dim}_{90}$ ) for pools 1 and 8, 7 and 14 days after fertilization.

most of the larvae from minor spawnings are small and had shell deformities, in shell formation, due to unripe or over-developed gametes.

In hermaphroditic pectinids, abnormalities can ensue from polyspermy or self-fertilization. It is the case for *Pecten maximus* where Beaumont and Budd (1983) noted slow growth rate among larvae issued from self-fertilization. Likewise they noted that eggs fertilized with sperm from a stripped gonad caused abnormality in about one third of the larvae. This last observation is similar to our studies where we noted that a stripped gonad can discharge unripe gametes. In this species ultrastructural observations on oocytes released from induced spawnings show prominent distortions. Apparently, normal oocytes can membranaceous lesions and cytostructural alterations (Dorange and

Le Pennec 1989). Subsequent fertilization of such oocytes has not yet shown larval abnormalities.

During gametogenesis holding spawners at 14°C induces alterations which either favor the formation of abnormal larvae or introduce a gap in growth, regardless of any growth potential assessed by the  $\text{Dim}_{90}$ . Considerable heterogeneity in the distribution of lipid reserves in ripe oocytes demonstrated by Dorange et al. (1989) could explain these observations. Likewise, Gallager and Mann (1986) observed that initial egg lipid content of *Ostrea edulis* is closely connected to the larval survival of both straight hinge and pediveliger stages. High survival is more generally accompanied by a high lipid index, but a high lipid index does not guarantee good survival (Gallager et al. 1986). Helm et al. (1973) also concluded that viability of

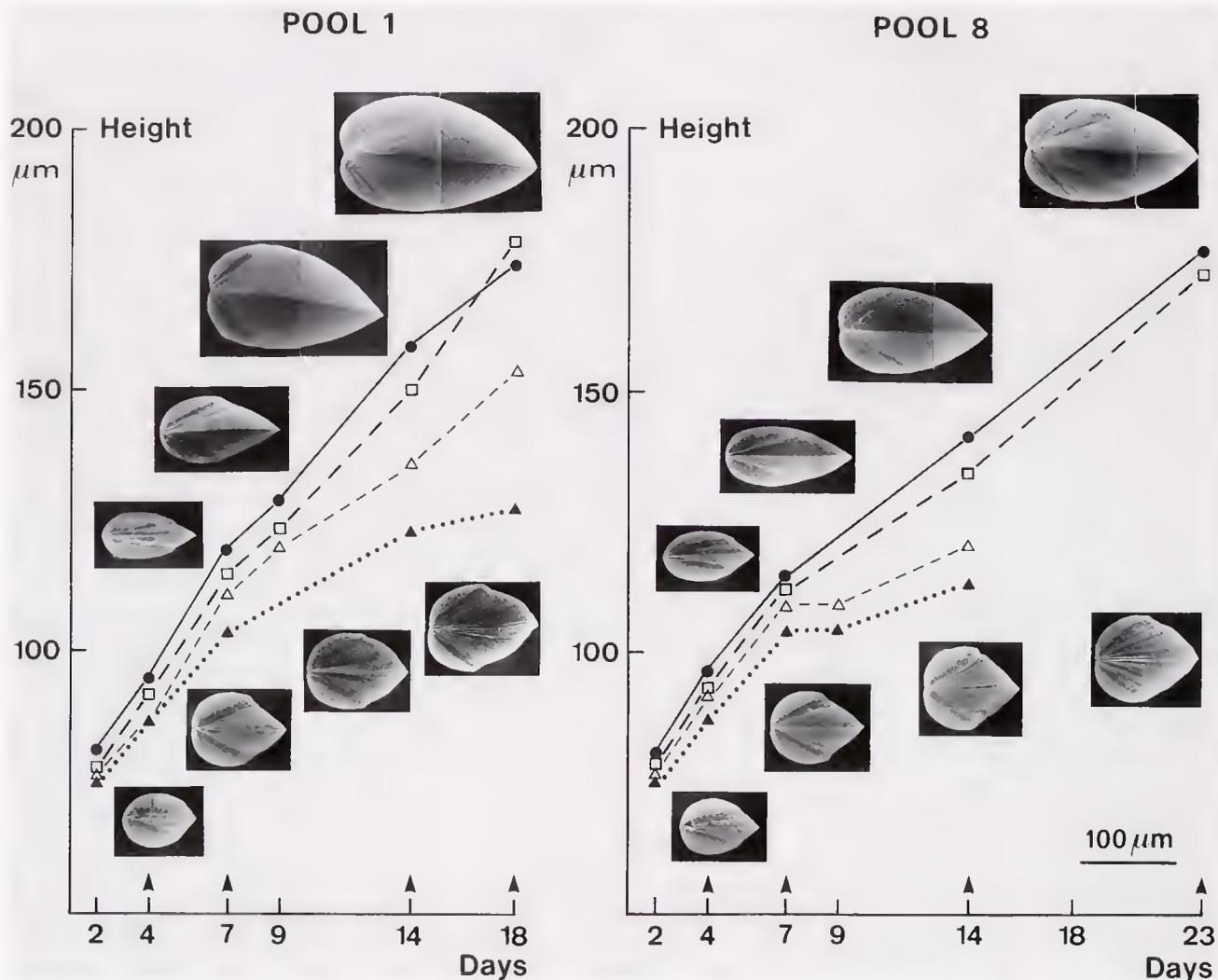


Figure 4. Larval growth for different  $Dim_{90}$  classes in pools 1 and 8. Shown are typical shells from individuals with lowest  $Dim_{90}$  ( $<52 \mu\text{m}$ ) and greatest  $Dim_{90}$  ( $>60 \mu\text{m}$ ). CL 1:  $Dim_{90} \leq 52 \mu\text{m}$  (shells above the curves), CL 2:  $52 \mu\text{m} < Dim_{90} \leq 56 \mu\text{m}$ , CL 3:  $56 \mu\text{m} < Dim_{90} \leq 60 \mu\text{m}$ , CL 4:  $Dim_{90} > 60 \mu\text{m}$  (shells below the curves). NB: The age of the larval shells is indicated by the arrows.

TABLE 2.

The mean height of larval shell (H) in the four  $Dim_{90}$ -classes in pools 1 and 8 at days 2, 7 and 14 and results of statistical analysis (t-test).

Days	CL	Pool 1				H	SD	Pool 8				Days
		N	SD	H	H			SD	N	CL		
2	+++	<52	105	3.25	81.14	NS	80.36	3.95	47	<52	2	
	+++	52-56	135	3.74	77.86	+	79.03	3.35	102	52-56		
	+	56-60	100	3.08	76.06	+	76.95	2.69	102	56-60		
		>60	67	2.76	75.01	NS	74.57	2.70	106	>60		
7	++	<52	32	6.10	119.41	+	114.47	6.69	14	<52	7	
	++	52-56	32	5.29	114.57	NS	112.16	6.71	13	52-56		
	+++	56-60	36	4.65	110.78	NS	109.02	5.50	28	56-60		
		>60	31	6.57	103.54	NS	103.47	6.22	32	>60		
14	NS	<52	33	15.78	158.49	+++	141.31	21.07	24	<52	14	
	++	52-56	58	21.34	150.19	+++	134.86	19.06	49	52-56		
	+	56-60	36	20.61	135.90	+++	120.98	13.37	44	56-60		
		>60	18	20.07	122.92	+	113.23	14.32	41	>60		

SD: standard deviation, N: number of measurements, NS: non significant, +:  $p < 0.05$ , ++:  $p < 0.01$ , +++:  $p < 0.001$ .

*Ostrea edulis* larvae is significantly corrected with their lipid content and more particularly with the neutral lipid fraction at the time of release. We suppose that larvae issued from delayed spawnings use up vitellin reserves more quickly.

Deformities of the prodissoconchs disclosed by the  $Dim_{90}$  can also be combined with velum abnormalities or functional difficulties in the capture of algal cells. Strathmann et al. (1979) and Cragg (1980) noted that *Pecten maximus* larvae have a lower clearance rate when the velum is poorly developed. Thus in the rearing tanks slow growing larvae were less colored, a feature considered to be related to the quantity of ingested algae (Loosanoff 1966; Le Penneec and Rangel 1985). The feeding deficit might accentuate the slowdown of growth observed after the day 7 in pools 1 and 8, particularly at the end of the mixotrophic stage (Lucas et al. 1986).

In the natural environment, numerous parameters influence bivalve recruitment, principally viability of emitted gametes, gamete and larval dispersion, feeding and predation of larvae and effects of poisons (Thorson 1946, Bayne 1976, Vance 1973a, b, Sprung 1988, Boucher 1985, Carriker 1986, 1988, Salaün 1987). After those experiments, we concluded that the quality of larvae, depending on the gametes quality, partially determine recruitment. In our cultures, the larvae descended from the spawnings (pool 1; constituted with the production of five genitors) obtained in

phase with the second spawning in the Bay of St Brieuc gave the best metamorphosis rate. In the field, these larvae should ensure a good recruitment if exogeneous conditions are favorable.

$Dim_{90}$  measurements allow the estimation of growth both in the natural environment and hatcheries. *In situ*, this additional index permits a differentiation of cohort larvae and a better analysis of larval spatial movements. It is to be used in parallel with hinge analysis which ages larvae from the first larval week (Salaün et al. 1989). Both indices permit the differentiation, among small larvae, or those individuals which just appeared in the plankton from larvae with a high  $Dim_{90}$  characterizing a low growth potential; indeed, such abnormal individuals can remain alive in a hatchery up to fifteen days, even without food (Salaün, unpublished data). *In situ*, the impact of exotrophy on larval development could be estimated by following the increase of height shell for larvae showing a high growth potential, that is a low  $Dim_{90}$ .

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## COMPARATIVE STUDY OF GROWTH AND SURVIVAL OF TWO COLOUR MORPHS OF THE CHILEAN SCALLOP *ARGOPECTEN PURPURATUS* (LAMARCK 1819) IN SUSPENDED CULTURE

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**ABSTRACT** During an 11 month study, growth and survival of an uncommon yellow colour morph was compared with that of the common brownish-purple morph of *Argopecten purpuratus* in suspended culture. The yellow morph had a significantly lower absolute growth rate, a lower value of the growth performance index and lower survival rate. These results suggest a genetic association between yellow colour and a lower general performance under normal environmental conditions. Possible explanations are discussed.

**KEY WORDS:** scallops, shell colour, growth, survival, aquaculture

### INTRODUCTION

Natural populations of the scallop *Argopecten purpuratus* are composed of a wide spectrum of colour morphs including white, yellow, reddish purple and brown, and it appears that the pattern of colouration differs slightly between populations (Vildoso and Chirichigno 1956, Wolff 1985). No study has been carried out to quantify colour morph distributions, evaluate their genetic basis or assess possible relationships between shell colour and population parameters such as growth and survival rates. In studying the colour morph distribution of the Argentinian scallop, *Chlamys patagonica*, Waloszek (1984) found a significantly higher percentage of yellow specimens in areas where the average body size was smaller, and hypothesized that overall growth might be inferior in the yellow colour morph. Yellow colour morph of *A. purpuratus* comprise about 1–3% of populations in Peru (Wolff 1985) and Chile (pers. observation) but in the hatchery of the "Universidad Católica del Norte," Chile, they occur in proportions of 8 to 15% (Garrido, personal observation, 1989). This might be due to the absence of selective forces in the hatchery allowing certain genotypes to survive that might otherwise be eliminated in nature.

Based on this assumption and the above cited observation of Waloszek (1984), we decided to conduct a study to compare growth- and survival-rates of the yellow colour morph with the common "normal" brownish-purple colour morph of *A. purpuratus*.

### MATERIALS AND METHODS

Yellow and normal (brownish-purple) coloured juvenile scallops, 20–30 mm shell height, were taken from spat collectors in Tongoy Bay in June, 1987 and transferred to the laboratory, where they were held in tanks with running sea water. A sample of forty-five scallops of each colour morph was measured to the nearest 0.1 mm using a caliper. For subsequent monitoring of growth and survival, the specimens were tagged with a numbered plastic disc at-

tached with epoxy cement to the upper valve and put into pearl nets; 3 pearl nets per group, each containing 15 scallops. The nets were hung from a longline at a depth of 6 m near the university pier in Herradura Bay (Fig. 1). Nets with yellow morphs were alternated with those of normal coloured scallops to intersperse replicate groups along any potential environmental variation.

The pearl nets were taken out of the water at monthly intervals and all scallops were measured. Dead scallops were counted and removed. The pearl nets were cleaned of mud and epifauna, refilled at densities of 15 scallops each, and replaced in the sea. Dead specimens were replaced with living scallops of similar sizes of the same origin as the initial scallops.

An extra set of two pearl nets per group, each containing 25 scallops was hung from the longline to provide an independent assessment of survival rates at higher densities.

Water temperature was measured daily to assess its effect on growth rates.

### Growth

The height and wet weight increment (absolute growth rate) of each scallop was calculated for each monthly interval. These monthly increments were subsequently averaged for each pearl net and the standard deviation calculated. After testing for homogeneity of variances of the group means, the differences in growth rate between pearl nets of the same colour group were tested using an analysis of variance. Since no differences occurred, the data for each group (yellow and normal specimens) were pooled for subsequent analysis.

Four approaches were chosen to assess differences in growth between both groups. The first consisted of calculating absolute growth rates from average increments in shell size and weight per month.

Approaches two to four were based on the assumption that growth in both groups can be described by the von Bertalanffy growth curve as was found for the same species

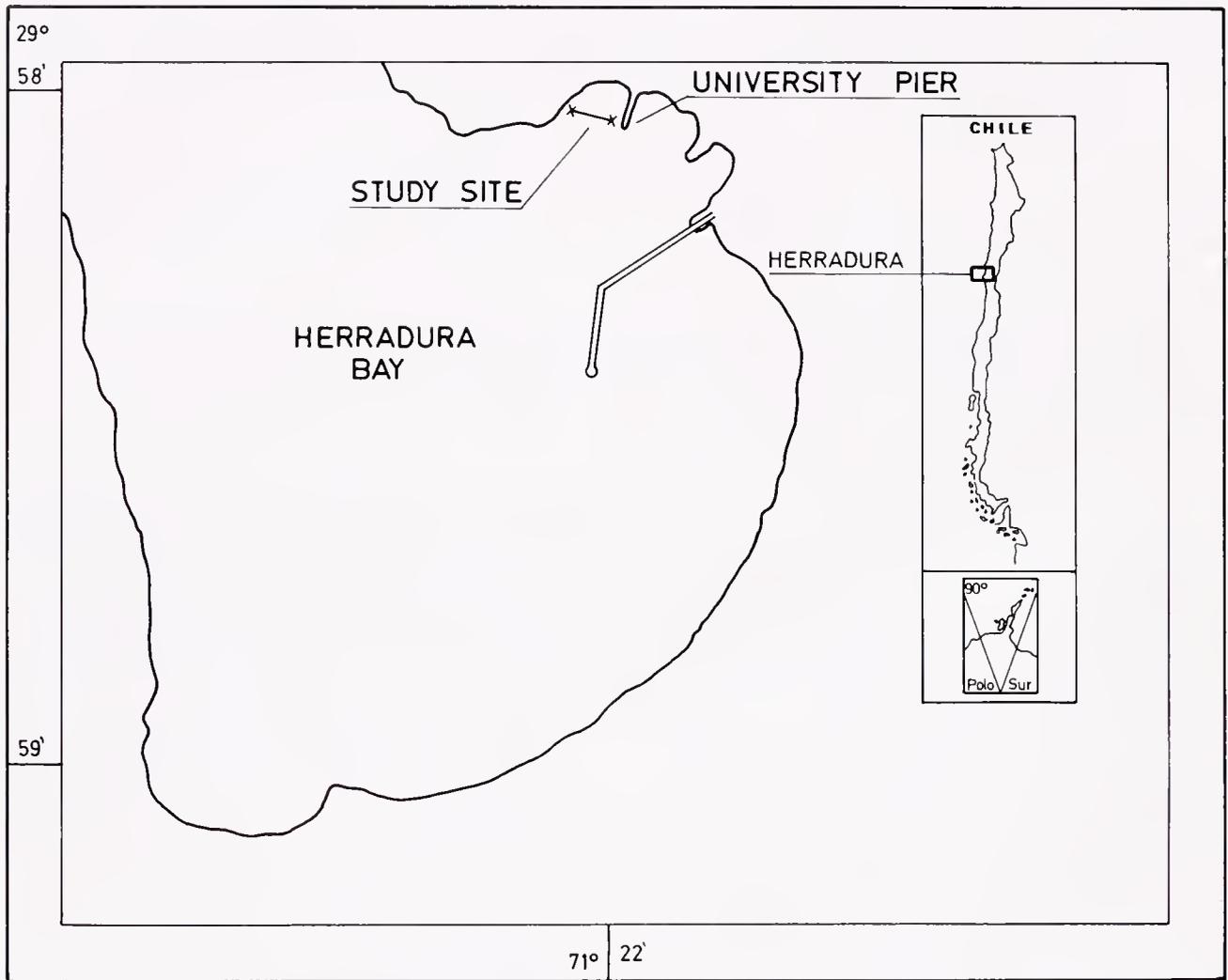


Figure 1. Map of Herradura Bay with study site.

in Peruvian waters (Wolff 1987). For the second approach, the instantaneous growth rate  $k$  and the asymptotic length  $L^\infty$  (von Bertalanffy 1934) were calculated using a model developed by Munro (1982) as follows:

$$\log(L^\infty - L_1) - \log(L^\infty - L_2) = k(t_2 - t_1),$$

where

$L^\infty$  = asymptotic length in the von Bertalanffy growth equation

$k$  = instantaneous growth rate

$L_1, L_2$  = shell sizes at times  $t_1$  and  $t_2$

The procedure was as follows: a trial value of  $L^\infty$  was inserted and  $k$  was estimated for each interval. The  $k$ -values of each interval were then averaged and the coefficient of variation calculated. The procedure was repeated with new values of  $L^\infty$  until the coefficient of variation of the average  $k$ -value reached a minimum. As this model does not allow for calculation of the third parameter of the von Bertalanffy

growth equation " $t_0$ " (theoretical age at length zero), this value had to be calculated in a different way. For this study we assumed an age of approximately two months at a shell size of 20 mm (DiSalvo et al. 1984) for both groups and calculated the  $t_0$ -value by reordering the von Bertalanffy-growth equation:

$$t_0 = t + (1/k) \ln(1 - L_t/L^\infty),$$

where

$t_0$  = theoretical age at length zero

$t, k, L_t$  and  $L^\infty$  as explained above

The third approach was to assess the effect of temperature on the instantaneous growth rate  $k$ . In order to do this, the monthly  $k$ -values were calculated, plotted and compared with the graph of monthly temperatures.

Finally, the growth performance index  $O'$  (Pauly and Munro 1984) was calculated for each group to estimate the

difference in growth capacity between the two groups using the following equation:

$$0' = \log k + 2 \log L^\infty,$$

where  $k$  and  $L^\infty$  are as defined above.

#### Survival

The number of dead individuals was recorded in each pearl net at monthly intervals and an average monthly survival rate calculated for both groups. The same was done for the extra set of pearl nets containing 25 scallops at the start of the study.

#### Morphometric Relations

To determine morphometric differences between the two groups existed, regression analyses of any two shell dimensions (height, length and width) within a group were calculated using the following equation:

$$L_x = a + b L_y,$$

where  $L_x$  and  $L_y$  are any two shell dimensions (cm).

Height, length, and width were also plotted against wet weight using the allometric equation:

$$W = a * Lx^b,$$

which was transformed logarithmically to:

$$\log W = \log a + b \log Lx,$$

where  $W$  represents wet weight (g) and  $Lx$  stands for any one shell dimension (cm).

For all data pairs geometric mean regressions were calculated and the differences between the regression coefficients of the yellow and normal specimens determined.

#### RESULTS

Yellow and normal coloured specimens grew at significantly different rates (Fig. 2a, b). The final differences in shell height and weight were 23.1 mm and 53 g, compared to initial differences of 2.8 mm and 1.8 g respectively. Figure 3 shows the von Bertalanffy growth curves for both groups as derived from the growth increment data and gives the corresponding growth equations.

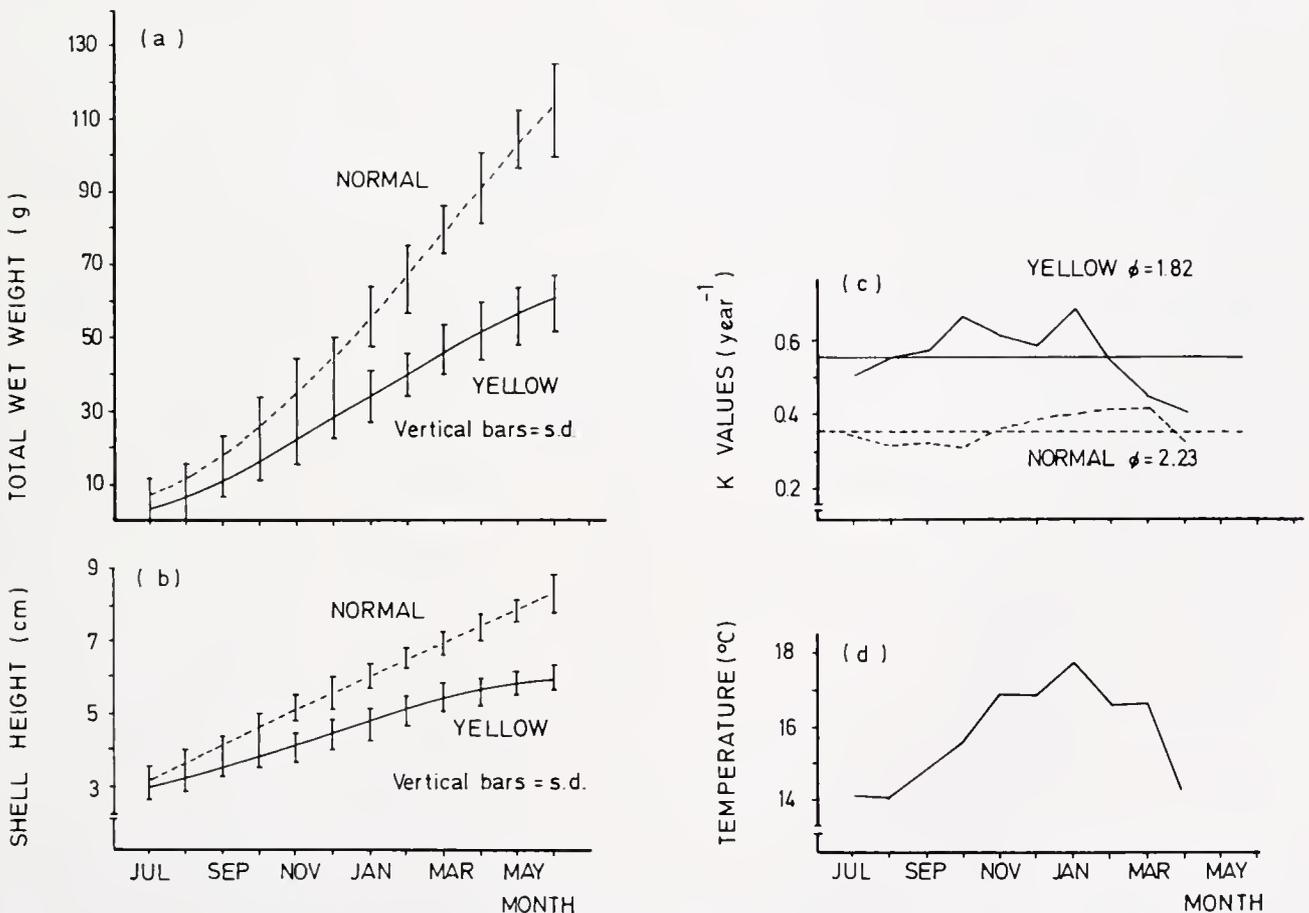


Figure 2. (a) Wet weight increments, (b) shell height increments, (c) average and monthly k-values and growth performance index "0'" of yellow and normal specimens of *Argopecten purpuratus*, and (d) water temperature in Herradura Bay during the study period, July 1987–June 1988.

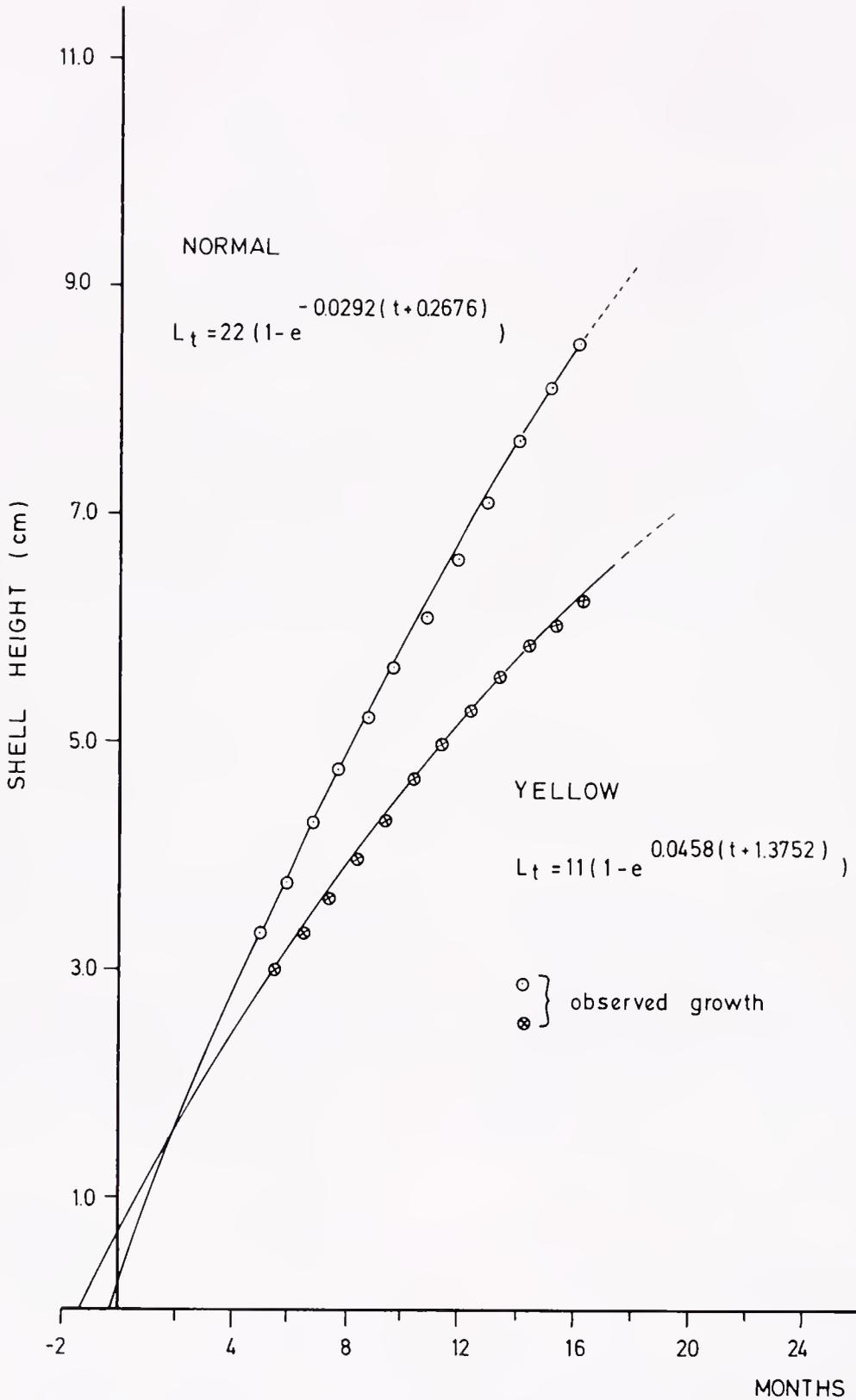


Figure 3. Von Bertalanffy curves and growth equations for yellow and normal coloured morphs of *Argopecten purpuratus* as derived from the shell height increment data.

The growth performance value—of normal specimens was 25% higher than that of the yellow scallops (2.23 compared to 1.82). The growth rate of the yellow morph was more variable and responded in a different way to temperature changes than that of normal coloured scallops (Fig. 2c, d). When temperature rose rapidly from October to November, growth rate decreased in the yellow morph but increased in the normal specimens. Temperature increases during December was more favourable for the yellow morph than for the normal specimens. When temperature dropped from January to February, growth rate decreased in the yellow morph but remained constant in normal specimens.

Survival was lower for the group of yellow morphs at all sampling intervals (Table 1). The average monthly survival rate was significantly lower (t-test,  $p < 0.001$ ) for the yellow scallops at both stocking densities (nets a, a\*, b, b\*, Table 1). The average monthly survival rate was 88.3% for the yellow morphs compared to 96.8% for the normal specimens (Table 1). No significant differences in % survival were found between nets of 15 and 25 individuals of the same colour morph.

Table 2 summarizes the equations of the geometric mean regressions calculated for the morphometric data for both groups. Differences between regression coefficients were significant for all regressions calculated. In general yellow morphs are rounder (height almost the same as length), deeper (shell width greater) and heavier than normal scallops (e.g., yellow scallops of 50 mm shell height are 50.9 mm in length, have a width of 19.7 mm and a weight

of 34.3 g, while normal scallops of the same height are 55.7 mm in length, have a width of 19.2 mm and a weight of 29.7 g). These differences are most probably due to the fact that yellow morphs are much older when compared with same sized normal scallops.

## DISCUSSION

Results of this study show a general inferior performance (slower growth and higher mortality) of the yellow colour morph compared with the normal morph. Growth rate of the yellow morph was also more variable and responded in a different way to water temperature changes (Fig. 2c, d).

Wolff (1987) found the growth rate of *Argopecten purpuratus* in Pisco, Peru to be strongly related with spawning cycle, being lowest after periods of peak spawning. According to Akaboshi & Illanes (1983) spawning of *A. purpuratus* peaks during spring and summer in Tongoy Bay. It could thus be hypothesized that the drop in growth rate of the yellow morphs during October and November (spring), when temperature was rising (Fig. 2c, d), was due to spawning during this period. A second spawning of the yellow morph may have occurred after the temperature maximum in January (summer) when growth rate and temperature dropped.

Hoagland (1977) and Newkirk (1980) infer that light shell colourations in molluscs absorb less light and heat and could represent warm water adaptations. The latter author reports that within each population and year class of *Mytilus edulis*, the unusual brown morph remains 10–20%

TABLE 1.

Survival of yellow and normal coloured Chilean scallops, *Argopecten purpuratus*, during the study period, August 1987–July 1988.

Months	% Survival					
	a	Normal b	[(a + b)/2]	a*	Yellow b*	[(a* + b*)/2]
Aug.	100	100	100	94	93	93.5
Sept.	96	90	93	85	86	85.5
Oct.	97	92	94.5	88	91	89.5
Nov.	94	100	97	83	93	88
Dec.	93	96	94.5	90	81	85.5
Jan.	96	100	98	92	100	96
Feb.	97	88	92.5	85	73	79
Mar.	95	100	97.5	93	83	88
Apr.	99	100	99.5	92	91	91.5
May	99	100	99.5	86	83	84.5
June	96	100	98	80	100	90
July	—	96	—	—	80	—
X	96.5	96.9	96.8	88.0	88.6	88.3
s.d	2.2	4.7	2.7	4.6	8.3	4.7

t-test for paired means:

a, a\*:  $t = 6.29$  (d.f. = 10),  $p < 0.001$ , a, b\*:  $t = 4.59$  (d.f. = 11),  $p < 0.001$  (a + b)/2, (a\* + b\*)/2:  $t = 7.82$  (d.f. = 10),  $p < 0.001$

a, b:  $t = 0.22$  (d.f. = 11),  $p > 0.4$ , a\*, b\*:  $t = 0.19$  (d.f. = 11),  $p > 0.4$

a, a\*: averages of 3 nets, stocking density 15 each; b, b\*: averages of 2 nets, stocking density 25; X, s.d: averages and standard deviation for monthly survival rates.

TABLE 2.

Morphometric relationships between the variables shell height (H), shell length (L), shell width (W) and wet weight (Wt) as established by geometric mean regressions for the yellow and normal morphs of *Argopecten purpuratus* (dimensions in cm, weight in g).

Variables		Normal Scallops			Yellow Scallops			p (b-b*)
X	Y	r	b	a	r*	b*	a*	
H	L	0.9945	1.1204	-0.3100	0.9846	1.0153	0.1015	<0.01
W	L	0.9924	2.884	-0.3221	0.9859	2.5346	0.1854	<0.01
H	W	0.9889	0.3834	0.0344	0.9831	0.3945	-0.0047	<0.05
logH	logWt	0.9833	2.8300	-0.5047	0.9777	3.2363	-0.7264	<0.01

r, r\*: correlation coefficient; a, a\*: Y-intercept; b, b\*: regression coefficient (slope); p(b-b\*): statistical significance of the difference between regression coefficients of both groups.

smaller than the darker normal blue morph. For the same species Mitton (1977) states that survival at extreme temperatures appears to be the selective force that maintains polymorphism. The latter might hold true for *A. purpuratus*, since the yellow specimens occur at higher percentages in the hatchery of the "Universidad del Norte" where temperatures exceeds natural summer temperatures by about 4–7°C. In the present study, maximum growth and survival of the yellow morph occurred in January when water temperature was maximum (18°C), (Fig. 2c, d; Table 1).

Some literature suggests that the proportion of yellow colour morphs in natural populations of molluscs increases toward the limits of their distribution. Waloszek (1984) found higher percentages of yellow specimens of the Argentinian scallop *Chlamys patagonica* near the shelf edge. Hoagland (1977) reported a higher occurrence of yellow specimens of *Crepidula adunca* at the southern edge of its distribution in California. Beukema and Meehan (1985) reported high proportions of yellow shells of *Macoma baltica* in samples collected at the southern limit of their distribution in Europe. The findings of the latter two reports are consistent with the hypothesis that yellow morphs have higher temperature optima and should thus be expected to be more abundant in warmer waters. The observation of Waloszek (1984) is contrary to this hypothesis. The increased abundance of yellow morphs toward the limits of distribution of the above mentioned species could also possibly be the result of a reduced gene flow in these areas. If this is the case, the yellow scallops could represent genotypes of increased homozygosity with reduced general fitness. This hypothesis would also be consistent with the higher occurrence of yellow specimens in the hatchery where survival probability for inferior genotypes is higher

and where inbreeding is known to occur easily (Meffe 1986, Newkirk 1983). In this context it is interesting to refer to Wolff et al. (in press.) who found that when eggs were given a cold shock treatment (introducing artificially a "selection factor") no yellow morphs developed. All the juveniles produced were of relatively uniform brownish-purple colour.

Koehn and Gaffney (1984) showed that growth rate and "average metabolic efficiency" of *Mytilus edulis* was positively correlated with heterozygosity. Zouros et al. (1984) also found a highly significant positive relationship between multiple locus heterozygosity and growth rate in *Crassostrea virginica*. Cain (1988) stated that a general "low metabolic efficiency" could result in an inferior hardness of the shell, a feature he found to be characteristic for light colourations in bivalves. This observation coincides with reports of culturists from Tongoy Bay who observe that yellow specimens of *A. purpuratus* have weaker hinges and are more affected by the boring worm *Polydora* sp. The results of the present study show that the yellow colour morph of *A. purpuratus* is inferior for cultivation under normal conditions as its growth and survival rates are significantly lower than for the common brownish-purple morph.

Future studies should be made to assess the genetic basis for the yellow colour morph in *A. purpuratus* and to explain its persistence in natural populations.

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## HERITABILITY OF GROWTH RATE IN THE SOUTHERN BAY SCALLOP, *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY, 1822)

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**ABSTRACT** Realized heritability for growth rate in the southern bay scallop, *Argopecten irradians concentricus*, is estimated to be 0.206. This estimate is based upon the response to a single generation of selection in which a standardized selection differential ( $i$ ) of 1.525 standard deviations was employed, and a response to selection, also in standard units, of 0.314 was obtained.

Scallops employed in this research were collected in St. Joseph Bay, west of Apalachicola, Florida. A same age cohort was produced from a spawning of wild caught animals in October, 1987, at the Skidaway Shellfish Research Laboratory near Savannah, Georgia. Larval and juvenile progeny were reared in the laboratory for seven months, in ambient filtered sea water with food provided by Wells-Glancy cultured phytoplankton, before being placed in pearl nets within fiberglass tanks or a 0.1 ha pond for growout for 20 weeks. In order to retain all genetic variance for growth rate possible, care was exercised during all growth phases to prevent any size culling. Truncation selection of the most rapidly growing 15.9% (animals larger than one standard deviation above the mean) was carried out in October, 1988. A control parental group, identical in numbers to the select line group, was randomly selected from the entire population before isolation of the select parental group. Spawnings of the control and select parental groups occurred, respectively, in October and November of 1988. Animals were reared under the same conditions as the previous generation except that all progeny employed in the estimation of heritability were maintained in growout in a 0.1 ha pond for 10 weeks. At that time, September, 1989, control and select groups approximated the mean size of the parental group at the time of selection.

**KEY WORDS:** bay scallop, *Argopecten irradians concentricus*, growth rate, heritability

### INTRODUCTION

Use of the techniques of quantitative genetic selection in bivalve shellfish is still in its infancy. Work carried out in the 1970's, reviewed by Newkirk (1980) dealt primarily with heritability of traits in larval shellfish. The moderate to high heritability estimates, based for the most part upon parent-offspring regression and sib analysis, served mainly to provide evidence of exploitable genetic variance. Of greater usefulness to the shellfish breeder are studies of heritability of adult traits. The first published effort to select for growth rate in shellfish is that of Chanley (1961) with southern populations of the northern quahog, *Mercenaria mercenaria*. He compared growth over 15 months in the progeny of two large quahogs about 44 months of age, with growth in the progeny of one female and two male quahogs chosen randomly from wild stock. The two large quahogs were taken from a group of 120 of the most rapidly growing of the progeny from a single pair cross. After fifteen months, progeny of the large quahogs were about 60% larger than progeny of the randomly selected parents based upon a volumetric comparison. The number of crosses was very small, but the experiment strongly suggested that there were heritable aspects of growth rate. Several years ago, Michael Castagna (pers. comm.) at the Virginia Institute of Marine Science, initiated a multigeneration program of selection for growth rate in the northern quahog, which is still continuing. While not quantitative, the program is a modi-

fied form of mass selection. Preliminary results appear to be promising.

In several cases involving shellfish, the action of natural selection (Needler and Logie 1947) or of artificial selection (Haskin and Ford 1987, Beattie et al. 1980) has indicated the presence of significant genetic variance for disease resistance, but efforts to calculate the heritability of such have not been published.

Working with the European oyster, *Ostrea edulis*, Newkirk (1980) and Newkirk and Haley (1982, 1983) selected for weight at two years. While they found positive face value increases in weight in the progeny of selected animals relative to weight of control stock progeny, such differences were not significant. The authors noted that response to selection was less than expected, and suggested that inbreeding might have contributed to the lower performance of the selected groups (Newkirk and Haley 1983). In his 1980 paper, Newkirk also reported preliminary data provided by L. E. Haley on selection for growth rate in the American oyster, *Crassostrea virginica*, showing a positive response to selection in two year old animals.

In work with the blue mussel, *Mytilus edulis*, Mallet et al. (1986) employed sib analysis techniques to estimate heritabilities for growth and survival in larval, juvenile and adult mussels. Heritabilities estimated from half-sib groups for adult growth were remarkably different in two adult environments (0.92 and 0.22) and were statistically signifi-

cant. With respect to survival, additive variance estimates were found to be not significant.

In a recent effort with the northern quahog, *Mercenaria mercenaria*, Rawson and Hilbish (1990), using sib analysis methods, estimated the heritability of growth rate in juveniles at about nine months of age. In an experiment in which population density was controlled, heritability was estimated to be about 0.37.

Wada (1984, 1986) carried out genetic selection for shell width and shell convexity in the Japanese pearl oyster, *Pinctada fucata martensii*. After three generations of selection, realized heritabilities for rate of increase in both traits were calculated on the basis of regression of selection response on cumulative selection differential. That for shell width ( $h^2 = 0.467$ ) was somewhat greater than that for shell convexity ( $h^2 = 0.350$ ). As Wada pointed out, such heritabilities indicate that selective breeding for shell growth rate would be effective in pearl oyster hatcheries.

Hadley (1988) reported on the results of a selection program for increased rate of growth in the hard clam or northern quahog, *M. mercenaria*. To calculate response to selection, growth of the select line progeny was compared with growth in progeny of a "mean line" group of parents, equivalent in number to select line parents but selected from individuals about the mean rather than randomly from throughout the parental population. Of three experiments carried out, two yielded realized heritability estimates of 0.42 and 0.43. In the third experiment, a negative estimate ( $-0.10$ ) was obtained. Hadley indicated that this latter aberrant estimate may have been due to disparate rearing densities resulting from predation.

The bay scallop, *Argopecten irradians*, does not occur as a native on the Georgia coast. Wild caught scallops from Gulf populations have proved to be readily adaptable to the Georgia coastal area, and good progress working out the husbandry of this form has been achieved for the area in our laboratory (Walker et al. 1990). Efforts to determine heritability of growth rate in our captive population were initiated several years ago in the interests of estimating the rate of progress in decreasing time to harvest that might be expected of a program of selection for growth rate in scallops. Such a reduction of the time in and the expense of growout would obviously be of usefulness to the mariculturist.

#### MATERIALS AND METHODS

The scallops employed in this research were collected in St. Joseph Bay, west of Apalachicola, Florida on the northern coast of the Gulf of Mexico. They were transported within 24 hours after collection to the University of Georgia Shellfish Research Laboratory on Skidaway Island near Savannah, Georgia. Details of mariculture conditions and spawning protocols are described by Walker et al. (1990). In the cited paper, the genetic lines discussed below are designated Stock A.

In the fall of 1987, a number of scallop spawnings occurred spontaneously or were carried out producing cohorts of same-age animals that could be maintained to sexual maturity for purposes of initiating 1) lines selected for rapid growth and 2) control lines comparable in degree of inbreeding to selected lines. Immediately after capture on September 23, 1987, 230 live scallops, after transport to the Skidaway Shellfish Research Laboratory, were divided into a group of 150 which was placed in a conditioning environment at 18° to 20°C, and a group of 80 which was placed on a laboratory raceway at ambient temperatures ranging from 19° to 26.5° and averaging about 22.5°C. Both groups were provided with high food concentrations. On October 20, 1987, the group on the raceway underwent a vigorous, spontaneous spawning. Unfortunately, only a fraction of the zygotes produced could be salvaged because our facilities were strained at the time. While there is no evidence as to the precise numbers of individuals participating, the large number of animals on the raceway and the evidence of an active spawning event suggested that abundant genetic variation would be present in the sample of 720,000 fertilized eggs retained. These larvae were reared as a same-age parental cohort to provide adults from which an  $F_1$  line selected for rapid growth as well as an  $F_1$  control line were drawn. Larvae were reared in a 500 L tank, and subsequently in plastic containers of about 90 L for approximately 2 months at which time an estimated 10,445 were transferred to a raceway receiving ambient filtered seawater and Wells-Glancy cultured phytoplankton. During this period, larval densities were maintained at less than 1 per ml.

Particular care was taken during the egg and larval phases, as well as throughout the project, to avoid any type of culling. This was done in order to conserve all genetic variance that might bear upon rate of growth.

At slightly less than seven months of age on May 26, 1988 and at an average shell length of about 11 mm, juvenile scallops of this parental cohort were placed in three different land based systems for growout. Each system comprised 1000 scallops, distributed among 10 nets (replicates) at 100 animals per net (Walker et al. 1990). In each, scallops were suspended in pearl nets of 0.09 m<sup>2</sup> floor surface area in (1) a 4270 L fiberglass tank on a concrete pad at the Skidaway Island Shellfish Research Laboratory, Savannah, Georgia, (2) a 4270 L fiberglass tank buried in earth to within about 15 cm of the rim at the same site, and (3) a 0.1 ha pond containing penaeid shrimp at the Waddell Mariculture Center, Bluffton, South Carolina. After ten weeks, density of survivors was reduced by dividing the remaining scallops in each replicate into two sub-replicate nets of 40 to 50 scallops each. At nearly one year of age, on October 14, 1988, scallops were removed for shell length (ear-to-ear axis) measurements and survival determination (Table 1). Details of the culture systems employed are provided in Walker et al. 1990. ANOVA comparisons indicated that there were highly significant differences

TABLE 1.

Size attained by parental generation populations of the southern bay scallop on October 13, 1988 at fifty one weeks of age in different culture systems.

Site	Number Measured	Mean Shell Length in mm $\pm$ S.E.	Standard Deviation	Cut-off Point (mm)
Pad Tank	855	41.2 $\pm$ 0.13	3.87	45.1
Buried Tank	657	44.5 $\pm$ 0.18	4.67	49.2
Waddell Pond	170	42.8 $\pm$ 0.34	4.37	46.9

among the means, and each of the three groups proved to be significantly different from the others by Duncan's Multiple Range comparison (Walker et al. 1990). In order to conserve all genetic variance possible, scallops from all three culture systems were used to provide select and control parents in proportion to the total numbers in each group, making nearly 1700 animals available for the selection program.

Treating each culture system group separately, truncation selection was carried out to provide a cut-off point at one standard deviation above the mean shell length, thus providing for selection of the most rapidly growing 15.9% of each group (Fig. 1). The means of the selected parents for all groups were determined in standard deviations above the different group means and would be equal to one another because they represent identical proportions of their distributions. This composite mean, in standard units, represents the selection differential which is the intensity of selection ( $i$ ). It is employed as the denominator in the ratio that estimates realized heritability (Falconer 1981).

It is not practical with the bay scallop to employ the parental generation measurements at selection as a negative control in the determination of response to selection (the numerator in the ratio that estimates heritability) because environmental factors such as temperature, and food quality and quantity play a very important role in determining rate of growth. Accordingly, a randomly chosen sample from the parental generation cohort was employed as control line parents to produce an  $F_1$  generation that would be expected to exhibit similar growth to that of the parental generation under the same environmental conditions. Parents of control line progeny were randomly chosen prior to removal of the selected parental groups, but with each culture system group providing numbers in proportion to its own population numbers. In order to maintain equivalent levels of inbreeding in select and control lines, the number of parents chosen to produce the  $F_1$  control line was determined to be equal to the number of select line parents remaining after designation of the control line parents (Table 2).

Control and select line parental groups were maintained separately under identical conditions in a constant tempera-

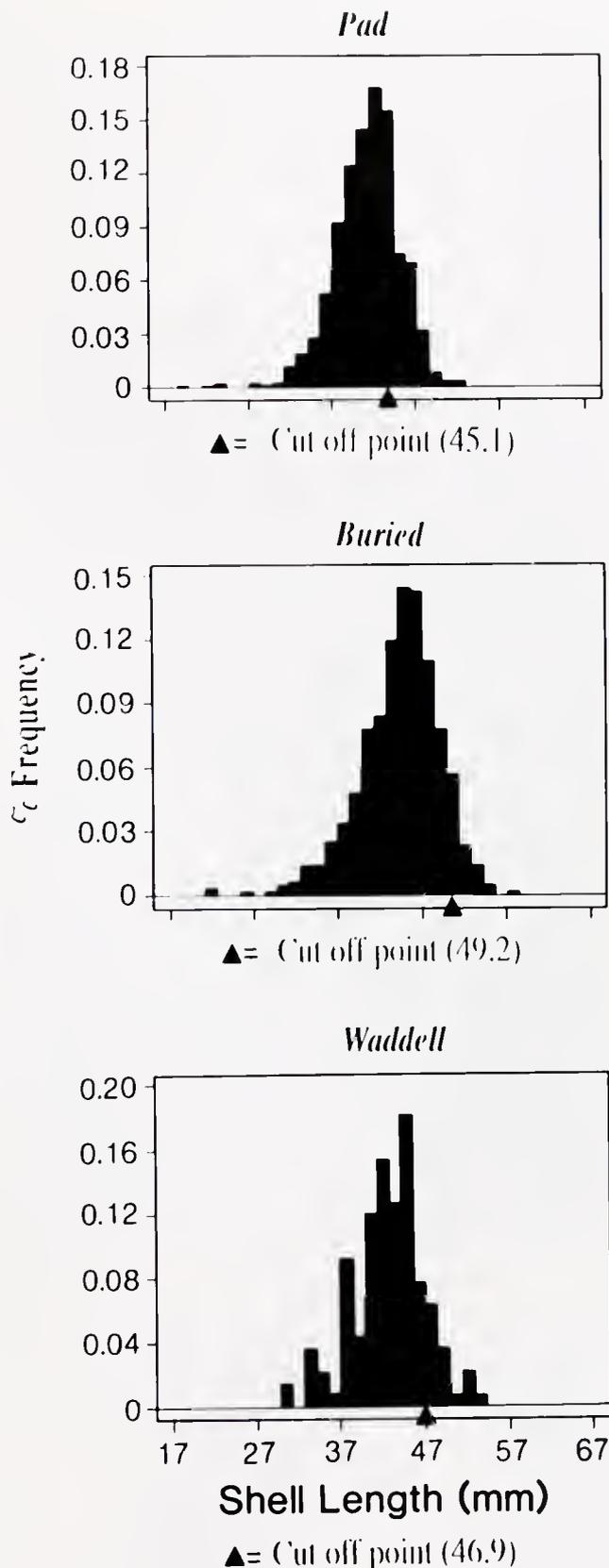


Figure 1. Size distributions and selection cut-off points of parental generation populations of the southern bay scallop in three culture systems.

TABLE 2.

Source of select and control line representatives of the parental generation in selection for increased rate of growth in the southern bay scallop.

Source	Number Available	Number Above Selection Threshold	To Control Line		
			To Select Line	From Above Threshold	From Below Threshold
Pad Tank	855	114	99	15	84
Buried Tank	657	103	87	16	71
Waddell Pond	143	22	19	3	16
Subtotals	—	—	—	34	171
Totals	1655	239	205		205

ture room at reduced temperature (18°–20°C) and high food concentration for conditioning. Prior to an attempt to induce spawning, the control parental group spawned spontaneously on Oct. 22, 1988, producing an estimated 2.5 million eggs. Subsequent spontaneous spawnings of select and control groups occurred on Oct. 30 and Nov. 11, 1988 (Table 3). In all cases larval densities were held at  $\leq 1/\text{ml}$  in tanks (500 L) from day two through the onset of metamorphosis, when all groups were transferred to raceway systems (340 L capacity). Raceways received ambient filtered seawater and Wells-Glancy cultured phytoplankton (Table 4). As with the parental generation, care was taken to prevent any size culling of  $F_1$  larval or post-set stages.

For both groups all spawnings appeared to be vigorous, involving many individuals, and we have assumed that the sizes of participating scallops are reasonably representative of the select and control parental group means.

It had originally been intended that we would focus first upon the  $F_1$  select and control progeny spawned on October 30, 1988, and reared in tanks, buried and on the pad, at the Skidaway Shellfish Research Laboratory for determinations of heritability of growth rate (Table 3). However, adequate food could not be provided for these groups and they did not attain the mean size of their parental population within a reasonable length of time (Table 5). Due to major construction at our facility, we were capable of providing for the  $F_1$  cohorts only about 50% of the amount of Wells-Glancy cultured phytoplankton that the parental generation had received during the growout phase (see Walker et al.

1990 for details). Fortunately, backup  $F_1$  select and control groups had been transported to the Waddell, South Carolina Mariculture Center for growout, and these groups developed at an acceptable rate (Table 5). Scallops were suspended in pearl nets of 6 mm mesh at densities of 50 per net on June 6 (Skidaway tanks) and on June 30, 1989 (Waddell Pond). All pearl nets were suspended in pairs, one above the other. The cohort at the Waddell Center were located in a 0.1 hectare shrimp pond similar to one in which some of the parental generation animals were maintained as described above. The ponds have a maximum depth of 1.2 meters, were aerated by a paddle wheel and contained penaeid shrimp during the scallop growout period. Scallop growth was determined at 10 and 20 weeks by measuring shell length of a sample of 30 per net with Vernier calipers. Means of measurements taken at 10 weeks, on September 8, 1989, approximated parental population means at the time of selection and so were employed in the computation of heritability for growth rate. It is important that response to selection be determined for  $F_1$  progeny at a stage of development comparable to that of the parental generation at the time of selection. Inspection of the data suggested that scallops in the upper nets were larger than those in lower nets. An ANOVA comparison of scallops in upper (surface) and lower pearl nets confirmed that scallops in the upper nets had grown at significantly greater rates than those in the lower tier for both controls ( $F_{1,208} = 9.29$ ;  $P < 0.0026$ ) and for select line animals ( $F_{1,208} = 7.27$ ;  $P < 0.0076$ ). Consequently, only scallops maintained in

TABLE 3.

Background information on the  $F_1$  scallop cohorts employed in this study.

Replicate Destination	Date Spawned	Egg Number	Postset Density	Postset Survival
Skidaway Tanks				
Control	10.30.1988	$2.38 \times 10^6$	130,000	0.009
Select	10.30.1988	$1.97 \times 10^6$	150,000	0.008
Waddell Pond				
Control	10.22.1988	$2.5 \times 10^6$	50,000*	0.066
Select	11.11.1988	$1.5 \times 10^6$	330,000*	0.002

\* Estimated density of pre-set larval stages at 11 days.

TABLE 4.  
Temporal aspects of the nursery stage of F<sub>1</sub> scallops.

Cohort	Raceway Phase	N(days)	Date Moved to Growout
Select Oct 30/88	Nov 8/88–Jun 6/89	(210)	Jun 6/89 to Skidaway tanks
Control Oct 30/88	Nov 8/88–Jun 6/89	(210)	Jun 6/89 to Skidaway tanks
Control Oct 22/88	Nov 7/88–Jun 30/89	(235)	Jun 30/89 to Waddell Pond
Select Nov 11/88	Nov 22/88–Jun 30/89	(231)	Jun 30/89 to Waddell Pond

upper nets were employed in the computation of heritability. Best estimates of heritability are obtained in environments that are optimal for growth and development.

The heritability computed here is an estimate of realized heritability based upon the ratio of selection response to selection differential. The selection differential expressed in standard deviations or intensity of selection (*i*) was discussed above. The selection response was based upon the difference between means of the progeny of select and control parents after attaining the approximate size of the parental population at the time selection was carried out. The difference between select and control progeny means was converted into standard deviations, of the control progeny population, for estimating heritability. The methods employed have been detailed in Humphrey and Crenshaw (1989).

Data set comparisons were carried out by ANOVA using SPSS and SAS software.

## RESULTS

Mean shell length measurements of the three subpopulations of scallops employed in selecting for growth rate are provided in Table 1 and graphed in Figure 1. These three subpopulations were reared under different growout systems as described, but all were derived from a common spawning, and thus have a common genetic heritage. As indicated above, it was convenient to use as a cut-off point one standard deviation above the mean. Thus, truncation selection for the most rapidly growing 15.87% of each subpopulation was effected. Using the mean and standard de-

viation of each subpopulation the shell length cut-off points determined for the pad tank, the buried tank and the Waddell pond were, respectively, 45.1, 49.2 and 46.9 mm (Fig. 1). The means of selected portions of the subpopulations were estimated by employing the mathematical property of normal distributions that the mean of a selected group, falling under a truncated portion of the normal distribution, is equal to *z*, the height of the ordinate at the point of truncation, divided by the proportion of the total population being selected, i.e., the truncated portion of the distribution. The mean, in standard units, of all three selected populations is equal to *z* divided by the selected portion or:

$$\begin{aligned} \text{selected population mean} &= 0.242/0.1587 \\ &= +1.525 \text{ std dev} \end{aligned}$$

The means of the selected groups from the three subpopulations in mm, employing the mean and standard deviation of each, are, respectively as above, 47.2, 51.6 and 49.1 mm. The selected population mean in standard deviations, 1.525, translates directly into a standardized selection differential or intensity of selection (*i*). This indicates that the means of the selected populations are 1.525 standard deviations above the mean of the parental population from which they were selected. The proportionate allocation of individuals from each subpopulation to control and selected parental groups is shown in Table 2.

Response to selection is determined by subtracting the mean shell length of the F<sub>1</sub> progeny of the control line from the mean shell length of the F<sub>1</sub> progeny of the selected parents. This difference is then converted into standard de-

TABLE 5.  
Growth and survival of F<sub>1</sub> scallops reared in three growout systems.

Growout System	Initial Size mm ± s.e.	Size at 10 Weeks ± s.e.	% Survival at 10 Weeks ± s.e.
Skidaway Pad Tank			
Control	12.2 ± 0.24	30.1 ± 0.32	92.4 ± 1.5
Select	10.0 ± 0.26	29.1 ± 0.30	88.0 ± 1.6
Skidaway Buried Tank			
Control	12.2 ± 0.24	33.9 ± 0.32	91.0 ± 2.2
Select	10.0 ± 0.26	28.6 ± 0.37	80.4 ± 3.2
Waddell Pond			
Control	12.5 ± 0.20	43.3 ± 0.43*	75.0 ± 3.0
Select	14.0 ± 0.26	44.6 ± 0.37*	90.0 ± 2.0

\* ANOVA comparison,  $F_{(1,208)} = 5.12$ ;  $P < .02$ .

viations (of the control progeny distribution) for the computation of a realized heritability estimate. Measurements and pertinent statistics with respect to these two groups are given in Table 5. The difference between the means of the two groups is modest, 1.3 mm, but it is significant ( $F_{1,208} = 5.12$ ;  $P < 0.02$ ). In terms of standard deviations the difference is 0.314, and this figure represents an estimate of response to selection in standard units.

Realized heritability ( $h^2$ ) is equal to response to selection divided by the selection differential, and, in standard units:

$$h^2 = R/S = 0.314/1.525 = 0.206$$

#### DISCUSSION

This calculated heritability estimate may be interpreted to indicate that about 20% of the total phenotypic variance in growth rate is attributable to the average effects of genes. It is important to realize that a single estimate is meaningful, but estimates are quite variable due to both sampling errors and environmental variation, and it is desirable to repeat this experiment in successive generations for refinement of the estimate. It will also be instructive to carry out similar work with other populations.

Taking our estimate of heritability at face value, we have reduced the time to harvest by about 3 days with one generation of selection, based upon an average per day increment of 0.44 mm for the final 10 weeks period of growout (Table 5). It may be predicted that nine additional generations of selection at similar intensities would reduce time to harvest by about a month. We could increase the rate of selection progress, for example, by selecting as parents only scallops above the mean plus two standard deviations. But this would only increase the rate of progress by a bit over 50% because the mean of the selected tail does not increase directly in proportion with increases in the cut-off point. Further, the rate of decrease in heterozygosity would increase considerably with the increased intensity of selection. However, close monitoring of the select line for negative effects on fitness would permit detection of resulting problems and relaxation of selection in response to the problem.

From Table 5, it is apparent that growth rates of select and control populations in the Waddell Pond during the ten week growout period are very similar. Since we know that select line larvae have lower growth rates than controls (Heffernan et al. 1990), it is concluded that it was primarily during the postlarval juvenile stage, up to a shell length of about 14 mm, that select line progeny grew at a rate greater than that of controls. This greater overall growth of select line progeny occurred in spite of the fact that they were some 20 days younger than controls at the time measurements were taken (Table 3). We have ignored this age difference, approximately 8%, in the interests of following conservative procedure in estimating heritability. However, this factor is of considerable importance and our procedure

unquestionably tends to bias our estimate in the direction of significant underestimation.

It is possible to adjust our heritability estimate above by taking into consideration an additional twenty days of growth for the select group, to make the select group identical in age to the control group at the end of the first growout period. This procedure is flawed because there is a twenty day stretch at the beginning and at the end of this adjusted period of growth during which the controls and selects are not sharing the same environment. However, it is useful to calculate what may be considered an upper limit to a realized heritability estimate. We have growth data for the select scallop group for an additional ten weeks periods after the date of the heritability estimate, and so it is possible to estimate the growth that would have occurred in the first twenty days of the second ten weeks in growout. The mean growth of the select group over the full (second) ten weeks period was 6.39 mm, nearly one fifth that experienced during the first ten weeks (30.58 mm). This averages out to about 0.0913 mm per day, or about 1.83 mm for the first twenty days. Since growth rate was probably tapering off during this period, this is likely a conservative estimate. If we add the response employed in our original heritability ratio above (1.29 mm) to that due to an additional twenty days of growth (1.83 mm), we obtain an adjusted response estimate of 3.12 mm. This translates into 0.76 standard deviations and an adjusted heritability estimate:

$$h^2 = R/S = 0.76/1.525 = 0.498$$

While this estimate is most reasonably viewed as an upper limit estimate, we think it likely that further work will provide heritability estimates of growth rate in scallops closer to our upper limit than to our more conservative estimate.

We cannot rule out with certainty that the lower density of select line progeny, as compared with controls, during the nursery stage (on raceways) may have contributed to greater growth of the select line progeny. The density of the postset select line cohort was actually much greater than that of the control cohort at the outset. Numbers of the control cohort were 50,000 for the 340 L raceway, a density of about 0.15 larvae per ml, considerably less than the density, 0.5 larvae per ml, recommended by Bourne et al. (1989).

By comparison, the density of the postset select line cohort at the outset, 330,000 for the 340 L raceway, is equal to nearly 1.0 larva per ml., greater than the density recommended by Bourne et al. (1989) but still below the density of 4 or 5 per ml recommended by Castagna and Duggan (1971) and Castagna (1975). Presumably, if there had been an effect of this density differential, it would have been a negative effect on growth in the select cohort. By the time of transfer of both cohorts into pearl nets and growout, about eight months later, there had occurred a reversal of the relative densities of these cohorts resulting from consid-

erably lower survival in the select cohort resulting from than in the control population (Table 3). In the select cohort, a survival rate of 0.002 reduced the density to about 0.044 juveniles per cm<sup>2</sup> of raceway bottom by the time of transfer to pearl nets and growout, at a mean shell length measurement of 14.0 mm. By comparison, mortality in the control cohort on raceway (nursery stage) reduced the density to about 0.222 juveniles per cm<sup>2</sup> of raceway bottom by the time of transfer to growout, at 12.5 mm shell length. It is suspected, but not known, that most of the observed mortality occurred at and/or within eight weeks of setting. This is known to be a critical period and is often associated with heavy mortality (Castagna 1975, Bourne et al. 1989, Ó Foighil et al. 1990).

Duggan (1973) recommends an optimum density in growout of 25 scallops per ft<sup>2</sup> (~0.09 m<sup>2</sup>) of bottom area. However his results show very similar growth of 25/ft<sup>2</sup> (~0.09 m<sup>2</sup>) and 50/ft<sup>2</sup> densities up to shell lengths of about 35 mm. From results obtained in the Waddell Pond, we (Walker et al. 1991, In preparation) have confirmed Duggan's results, showing that in 6 mm mesh pearl nets at densities of 25 per (~0.09 m<sup>2</sup>), growth is slightly but significantly greater than at a density of 50 per (~0.09 m<sup>2</sup>) for both the first and second ten-week periods of growth. Our design called for densities of 50 per net (~0.09 m<sup>2</sup>) for control and select lines alike for the first 10 weeks to be reduced to 25 per net for the second period. However, as indicated above, it was decided to estimate heritabilities after the first period because mean control cohort size approximated that of the parental population at the time measurements were taken for purposes of establishing a selection cut-off point.

It is possible, but we think not likely, that the higher rate of growth of the select line cohort, relative to controls, may have been due in part to greater crowding of controls prior to growout. Such an effect would be a complicated one to evaluate inasmuch as it would have started, if indeed it did, as total scallop biomass reached a threshold level during development on the raceway and would have become increasingly effective as biomass per unit of space increased for whatever period of time remained before transfer to growout. We believe that adequate nutrition was provided for both select and control cohorts (nursery), and it is clear that scallop biomass per unit of floor space at the end of development on raceway was a fraction of that at the end of 10 weeks of growout in pearl nets.

Estimates of heritability of characteristics other than growth rate in shellfish would not be expected to be closely correlated with our estimate. Nor would growth rate heritability estimates based upon other methods of determination, e.g., sib analysis, or other life history stages be expected to be similar. Although a different species is involved, Hadley's (1988) results with the northern quahog (*Mercenaria mercenaria*) may be compared with our own. Ignoring her single negative estimate of realized heritability which

cannot be correct and which Hadley thought may have been due to disparate rearing densities, the other two,  $h^2 = 0.42$  and 0.43, are quite consistent and indicate that heritability of growth rate in that species is about twice as great as our conservative estimate for the southern bay scallop ( $h^2 = 0.206$ ) but a bit lower than our upper limit estimate ( $h^2 = 0.498$ ). If our conservative estimate proves to be more accurate, one interpretation of such a difference is that rate of growth in the short lived scallop, approximately one year to 18 months, may have been more critical and the focus of more persistent and intense natural selection than in the longer lived quahog, up to 46 years. Thus, the bay scallop may be thought of as an ecologically more opportunistic species, with genetic variance for and heritability of growth rate reduced by continuing selection for maximum growth rate.

The failure of the two Skidaway tank  $F_1$  cohorts, select and control, to grow at a reasonable rate, as well as the poor growth of the select line relative to controls in these tanks, is probably a direct result of undernourishment (Table 5). We know their daily food ration to have been 50% or less than that received by their parents at a comparable stage a year (= generation) earlier. Furthermore, the growth after 10 weeks for both select and control groups at the Waddell pond was much higher (30% for controls; 50% for selects) than for scallops in the Skidaway tanks, and there was a significant response to selection demonstrated by scallops in the Waddell pond. This focuses our attention on the importance of environmental factors in efforts to generate heritability estimates. It has been well documented that populations subjected to selection are often less hardy than unselected controls, a result largely attributable to inbreeding effects consequent to selection (Lerner 1954). It is also understood that selection carried out under one environment may (or may not) produce quite different results in another. Falconer (1981) discusses such genotype-environment interactions. It is also well documented that organisms which have been subjected to selection are prone to perform at a lower level than their control counterparts when stressful conditions prevail, as in the case of undernourishment in our Skidaway tanks. In a situation comparable to our own, it has been found that plants selected for high productivity during the "Green Revolution" effort often performed significantly more poorly than unselected stocks when not provided with the proper soil, optimum water and fertilizer available while the strains were being selected (Nebel 1981, Baer 1977). This should serve as a cautionary note for other workers in the field. The need to insure near "optimal" growout conditions for select and control lines is essential. Care should be taken to assess adequately environmental parameters (e.g., temperature, salinity and food availability) in the growout system chosen.

The fact that poorly fed selected line progeny actually grew more slowly, at face value, than controls would make

an attempt to calculate a realized heritability from these data a meaningless exercise, producing a negative heritability estimate.

We have provided results earlier that indicate a negatively correlated effect on larval growth rate in the progeny of the northern quahog selected for rapid growth rate to adult size (Heffernan et al. 1991). We also have data indicating significantly higher embryonic mortality by day 2 for the same progeny. Furthermore similar results have been obtained in studies of scallop offspring dynamics (unpub. data). Our interpretation of these effects is that arrays of genes responsible for growth rate to adult size are not identical to those determining larval growth rates. Thus, in focusing upon overall growth to the adult stage, we have lost a certain amount of larval rate of development and some embryonic survival as a cost of selection. However, the decreased larval growth rate is more than compensated for by increased juvenile and/or adult rate of growth.

There are a number of methods that may be employed to estimate heritability of a trait, each of which has its advantages, disadvantages and special implications. The method used in this research produces an estimate of realized heritability, i.e., an estimate of the response to selection that may be actually obtained. As pointed out by Falconer (1981), of the variety of methods for estimating heritability this method "provides the most useful empirical description of the effectiveness of selection . . ." The procedures involved in obtaining estimates of realized heritability by selection differential and response have been outlined and simplified with specific emphasis on shellfish by Humphrey and Crenshaw (1989). The protocol followed in this outline approximates closely the practices that the mariculturist breeder might follow.

An additional aspect of our approach that is appealing is that having maintained our populations of scallops for the two generations required to obtain an estimate of realized heritability, we have simultaneously made some selection progress toward the goal of shortening time in growout, and we are three years into selection research to reduce time to harvest for the bay scallop.

Several concerns are associated with our protocol, the most important of which include the assumptions of random breeding of parental stocks and equal gametic contributions of parents on the spawning table. It is clear that all broodstock present may not spawn, that there is likely great variation in the numbers of gametes produced by different contributors, and that early contributors to a given spawning event are likely favored in the extent to which they contribute to the progeny pool. The effects of natural selection and maternal effects may also lead to either relatively increased or decreased representation of specific parents in the progeny pool. However, in general, use of as large a number of potential parental animals as possible will make it most likely that animals that do spawn, or that

spawn early, will be not too distant from the mean, simply because of the numerical preponderance of animals about the mean. Although successive generations of selection will eventually lead to reductions in real heritability, as genetic variance is reduced, successive estimates based upon the first few generations of selection should serve to increase the reliability of realized heritability estimates.

With respect to variation in number of gametes produced by different individuals, it is a continuing concern that the small animals in any group will likely make lesser gametic contributions on the average than larger animals. The effect of this will be most important in the comparison of the progeny of select and control parents. Select parental animals will have no small members, but the control, having been randomly selected from the total parental phenotypic distribution, will include small representatives in the approximate proportion of their occurrence in the parental population. The effect of reduced productivity of small animals will be to make the heritability estimate an underestimate, (if the trait is heritable). If small members of the control group are not proportionately represented in the gamete and progeny pools resulting from a mass spawning, then the shell length mean of the  $F_1$  control progeny would be slightly larger than it would with proper representation of small animals, and the difference between the control and select means, the response to selection, would also be reduced. Since selection response is the numerator in the heritability ratio, if the selection response is biased downward, so will be the estimate of heritability.

Interestingly, if the progeny contribution of very rapidly growing (large) animals is reduced, the resulting effect will be in the direction of heritability overestimate. In general, it is expected that organisms about the mean will be more fit, i.e., will contribute more progeny to the next generation, than those at the extremes of a distribution (Wallace 1981). It is our feeling that our own conservative heritability estimate is probably an underestimate, but an estimate that will not be expected to mislead the shellfish breeder in his efforts to improve a shellfish stock.

Both natural selection and maternal effects may detract from the validity of heritability estimates based upon the selection differential and response ratio. We have no reason to believe that either has played an important role in our results. However, we have reduced the effects of natural selection to the greatest extent practical, and we expect to explore the importance of maternal effects by appropriate methods in the future. In the final analysis, the method employed here, even with its disadvantages, is clearly the method of choice in determining the effectiveness of selection.

Problems due to inbreeding and the consequent loss of heterozygosity will be expected to develop sooner or later in our select line. However, inbreeding in the parental generation, produced by wild caught animals, would be essentially non-existent. In the  $F_1$  generation limited inbreeding

would be expected, but reduced heterozygosity would be so slight as to produce negligible effects.

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## GEODUCK, *PANOPEA ABRUPTA* (CONRAD, 1849), SIZE, DENSITY, AND QUALITY AS RELATED TO VARIOUS ENVIRONMENTAL PARAMETERS IN PUGET SOUND, WASHINGTON

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**ABSTRACT** The relationship between geoduck, *Panopea abrupta* Conrad, 1849, size, density, and quality and several environmental parameters were examined from diver survey data from Puget Sound, Washington. The average shell length and whole wet weight of 11,154 geoducks were 135.2 mm and 872.2 g respectively. Shell length was inversely related to latitude and water depth. Average shell length of geoducks taken from mud, mud-sand, sand, and pea gravel-gravel substrates was 130.2 mm, 137.0 mm, 138.6 mm, and 128.8 mm respectively. Geoducks were contagiously distributed: the average number of clams per clump was 109 and the average number of clumps per 41.8 m<sup>2</sup> quadrat was 0.64. Geoduck density was inversely related to latitude and directly related to water depth and averaged 1.66 clams/m<sup>2</sup> throughout Puget Sound. Plants and animals most commonly associated with geoduck beds were chaetopterid polychaetes, sea cucumbers, sea pens, laminarian kelp, and other clams. Geoduck quality was inversely related to water depth.

**KEY WORDS:** geoduck, *Panopea abrupta*, size, density, quality, environment

### INTRODUCTION

The geoduck clam *Panopea abrupta* Conrad, 1849, is a large hiatellid which occurs from Alaska to Baja, California (Andersen 1971). It lives deeply buried in sand and mud substrates from the lower intertidal to depths of at least 110 m (Jamison et al. 1984). The clam spawns primarily in the late winter to early summer (Andersen 1971, Goodwin 1976, Sloan and Robinson 1984). They grow rapidly in prime habitats and can reach the average Puget Sound weight of 872 g in five years (Goodwin 1976, Goodwin and Pease 1987). Recruitment rates in many locations are very low (Goodwin and Shaul, 1984). Geoducks are very long-lived, reaching ages well over 100 years in British Columbia, Canada and Puget Sound, Washington (Shaul and Goodwin 1982, Harbo et al. 1983, Breen and Shields 1983, Sloan and Robinson 1984).

This large clam supports very important commercial fisheries in British Columbia and Washington State. Landings in British Columbia in 1984 were 3483 tons (Jamieson and Francis 1986) and peaked at 3922 tons in 1977 in Washington State (Goodwin and Pease 1987). The clams are taken by divers primarily in water depths of 6 to 18 m.

The Washington Department of Fisheries (WDF) routinely evaluates subtidal geoduck stocks by SCUBA diver surveys (Goodwin 1973, Goodwin 1978, Goodwin and Shaul 1978, Goodwin 1979, Goodwin 1980, and Goodwin and Shaul 1981). This paper presents the environmental data collected during the surveys and analyzes the relationships between this data and geoduck size, density, and quality.

### MATERIALS AND METHODS

A complete description of the methods will not be given in this paper. For this the reader is directed to Goodwin and Pease 1987. For this study Puget Sound was divided into

six regions (Fig. 1). These regions conform quite well to the divisions of the Sound used by oceanographers and are based on water circulation patterns and the location of sills (Strickland 1983, Collias et al. 1984).

Geoduck densities were estimated by diver counts of geoduck "shows" (siphons or marks left in the substrate by retracted siphons) along 0.91 m × 45.72 m transects. The transects were oriented with the long axis perpendicular to the shoreline and most were completed in water depths of 6 to 18 m. The counts were adjusted by a "show factor" to account for the geoducks that could not be detected by divers (Goodwin 1977). In most geoduck beds at any particular time a portion of the clams will have retracted siphons. If the holes in the substrate left by the retracted siphons fill in with substrate material, then the clams can be very difficult to detect by divers. The percentage of clams that can't be detected can be estimated by counting geoducks in nearby plots where the populations are known from previous work. This percentage, then, is the "show factor."

At the end of each transect the divers recorded the water depth, their subjective assessment of the surface substrate, and the presence of readily visible macroflora and fauna. The substrate was assigned to one or a combination of the following categories: mud, sand, pea gravel, and gravel. The boat operator recorded latitude and longitude at the start of each transect. At approximately 30% of the transects, a sample of ten or more geoducks was washed from the substrate with a water jet (dig samples). These samples provided data on geoduck size, quality, ease and speed of harvest, and substrate composition below the surface layer.

The greatest anterior-posterior length of the right valve was measured with calipers. Whole wet weight was measured after varying amounts of drainage time (few minutes to several hours). The quality of geoducks was evaluated

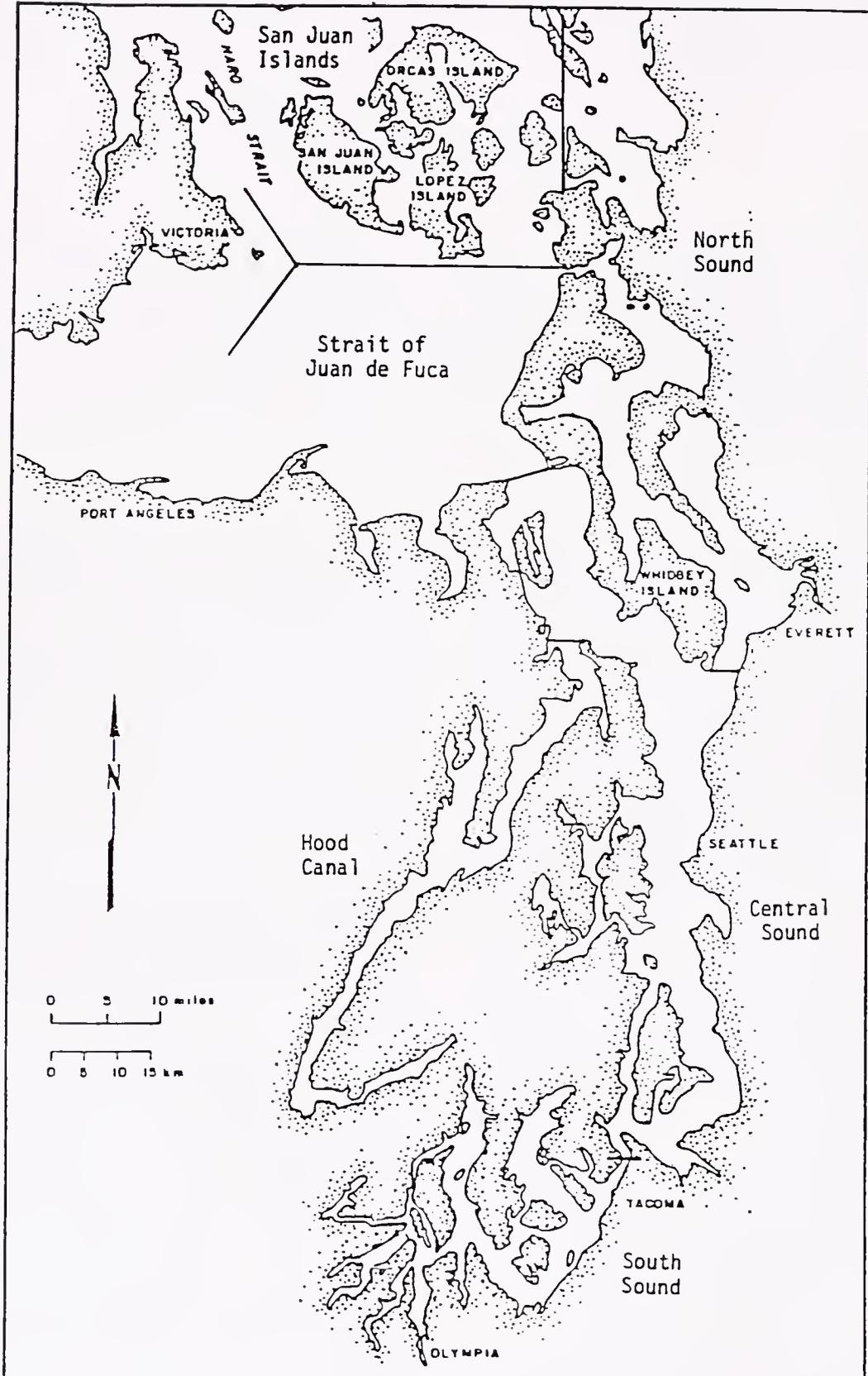


Figure 1. Six regions of Puget Sound.

by comparing the color of the meat in cross sections at the base of the siphon to permanent color standards. Geoducks were judged to be 1, 2 or 3 grade. Number one grade was the lightest color and three, the darkest.

The data were stored and processed on a Zenith 100 microcomputer. Most of the analyses were conducted with the Statpak version 3.2 statistical package from Northwest Analytical, Inc., Portland, Oregon. Some of the regression analyses were conducted with MINITAB on a Prime mini-computer.

The data were first analyzed by frequency histograms and tested for normality by the Chi-squared method. If the data proved to be normal, then standard parametric statistical tests were used (Zar 1974). If the data were not normal, then standard transformations (natural log, arc sine, and Taylor) were used to normalize it (Thoni 1967, Elliott 1971, Zar 1974) after which standard parametric tests were used. Data which could not be normalized were analyzed with non-parametric methods (Zar 1974). The probability level of significance in all multiple range tests discussed in this paper was 95%.

RESULTS

Numbers and Kinds of Samples Taken

The following results were based upon a maximum of 8698 transects and 2608 dig samples. The majority of those

samples were taken in the Strait of Juan de Fuca, Central Sound, Hood Canal, and South Sound due to the discovery of large numbers of geoducks in the early surveys in these regions.

Geoduck Length and Weight

Overall geoduck length and weight frequency distributions and descriptive statistics for these distributions are shown in Figures 2 and 3. The average length and weight of 11,154 geoducks measured was 135.2 mm and 872.2 g. The range of length and weight varied from 49 mm to 212 mm and 28 g to 3250 g. The low number of small geoducks shown in the figures is due partly to the fact that the water jet method is not an efficient system to obtain small clams. The distributions of sample means were tested for normality. Mean geoduck shell lengths were normally distributed (Chi square = 11.49, P > 0.05). Therefore the length data was not transformed and parametric tests were used in the analyses involving length. Mean geoduck weights were not normally distributed (Chi-square = 87.1, P > 0.05). Natural log transformed mean weights were normally distributed (Chi-square = 17.6, P > 0.05) and used in parametric tests. Several factors may have contributed to the non-normality of the weight data. Whole wet weight could have been affected by the length of time between sampling and weighing (drain time) which varied from day to day. Weight may have been affected by the harvest with a water

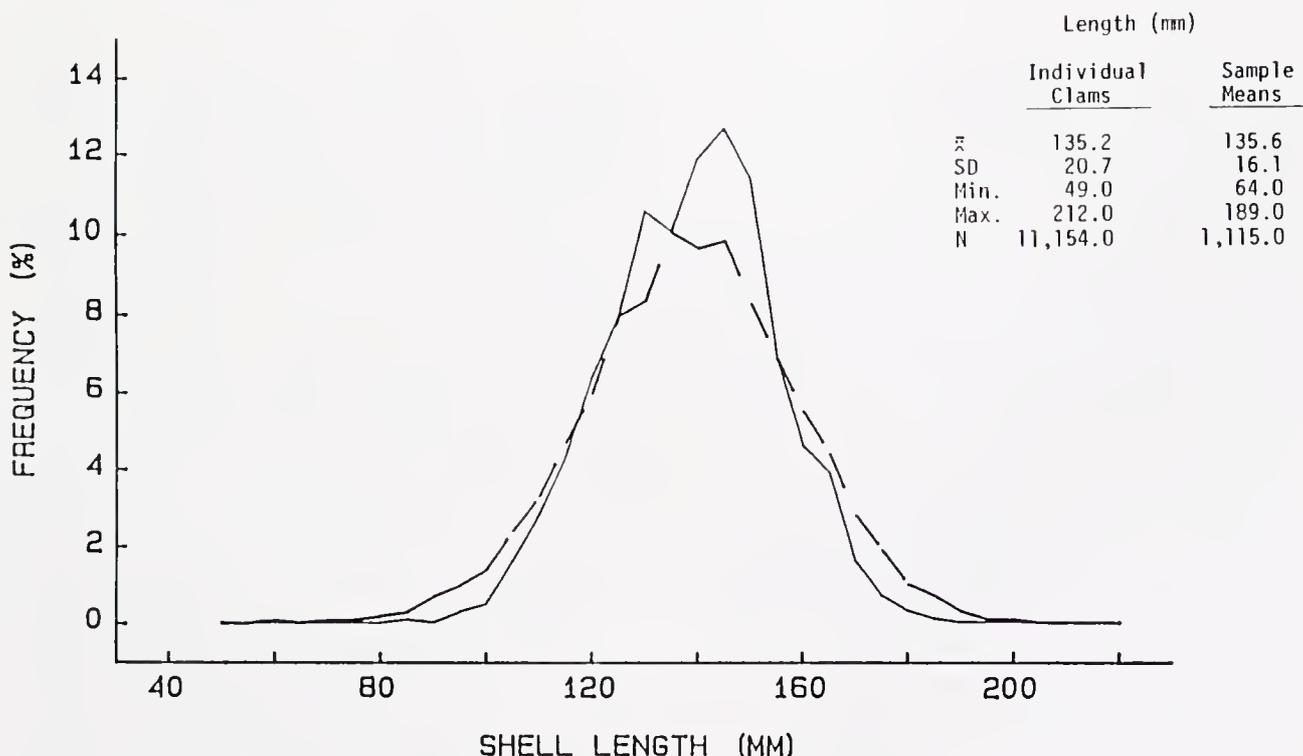


Figure 2. Length frequency distribution of individual geoducks and sample means (see summary statistics in Table 3) from 1115 samples collected from Puget Sound between 1973 and 1985. Solid line = sample means and broken line = individual clams.

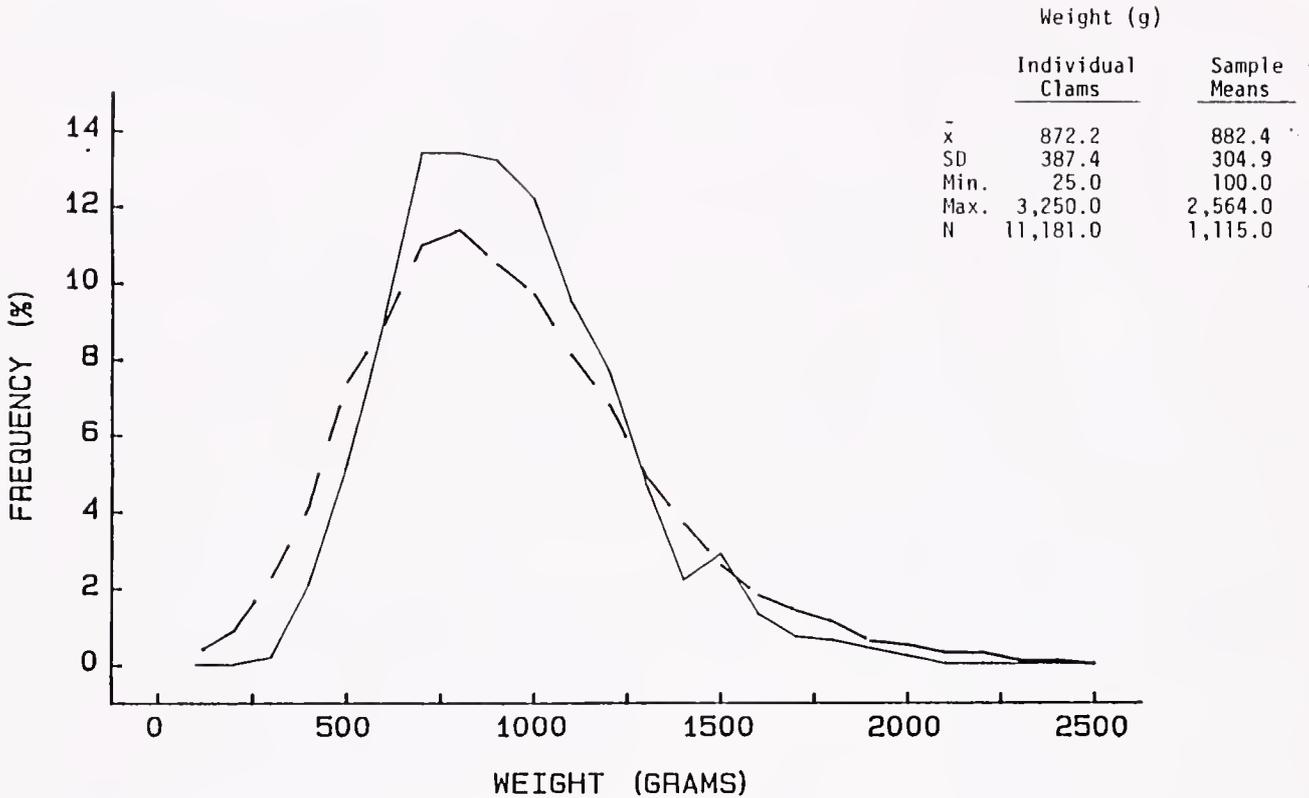


Figure 3. Weight frequency distribution of individual geoducks and sample means (see summary statistics in Table 3) from 1115 samples collected from Puget Sound between 1973 and 1985. Solid line = sample means and broken line = individual clams.

jet. Geoducks were sometimes hit by the water jet, resulting in damage or loss of soft body parts and body cavity water.

#### Length and Weight Versus Geographic Area

The length and weight (non-transformed data) of geoducks from four regions of Puget Sound are 144.7 mm and 1070.9 g, 136.4 mm and 899.9 g, 131.6 mm and 776.0 g, and 134.4 mm and 704.9 g for South Sound, Strait of Juan de Fuca, Central Sound, and Hood Canal respectively. The significance of the difference between the mean size in the different areas was assessed with a one-way analysis of variance (ANOVA) and the Newman-Keuls multiple range test (Zar 1974).

The ANOVA showed a significant difference in mean length and weight (transformed data) between regions (length  $F = 102.9$ ,  $P < 0.001$ ), (weight  $F = 116.8$ ,  $P < 0.001$ ). The Newman Keuls multiple range test showed that the mean size in each region was significantly different from the mean size in all other regions.

#### Length and Weight Versus Latitude

In the previous section, significant differences in length and weight of geoducks were demonstrated in various regions of Puget Sound with the largest clams found in the

South Sound region. A regression analysis established a significant relationship between latitude and geoduck shell length (Fig. 4). The best fit is a log linear model with a slope significantly less than 0 ( $F = 128.8$ ,  $P < 0.001$ ). In other words, in Puget Sound geoduck length decreases significantly along a south to north gradient. A regression analysis of latitude versus natural log-transformed weight showed the same results ( $F = 107.0$ ,  $P < 0.001$ ).

#### Length and Weight Versus Water Depth

The geoduck length and weight data were placed into three groups based on water depth; those taken in water less than 9.1 m, those from 9.1 m to 13.7 m, and those from depths greater than 13.7 m. The average shell length and whole wet weight (non-transformed) of geoducks taken from these water depths are 146.2 mm and 1078.8 g, 135.5 mm and 875.0 g, and 125.8 mm and 707.7 g respectively.

Differences between the means of these three groups for length and weight (transformed data) were assessed with a one-way ANOVA and were found to be significant (length  $F = 97.6$ ,  $P < 0.001$ ; weight  $F = 100.5$ ,  $P < 0.001$ ). The Newman-Keuls test revealed a significant difference between each depth group.

A regression analysis of water depth and shell length showed a significant ( $F = 253.7$ ,  $P < 0.001$ ) inverse rela-

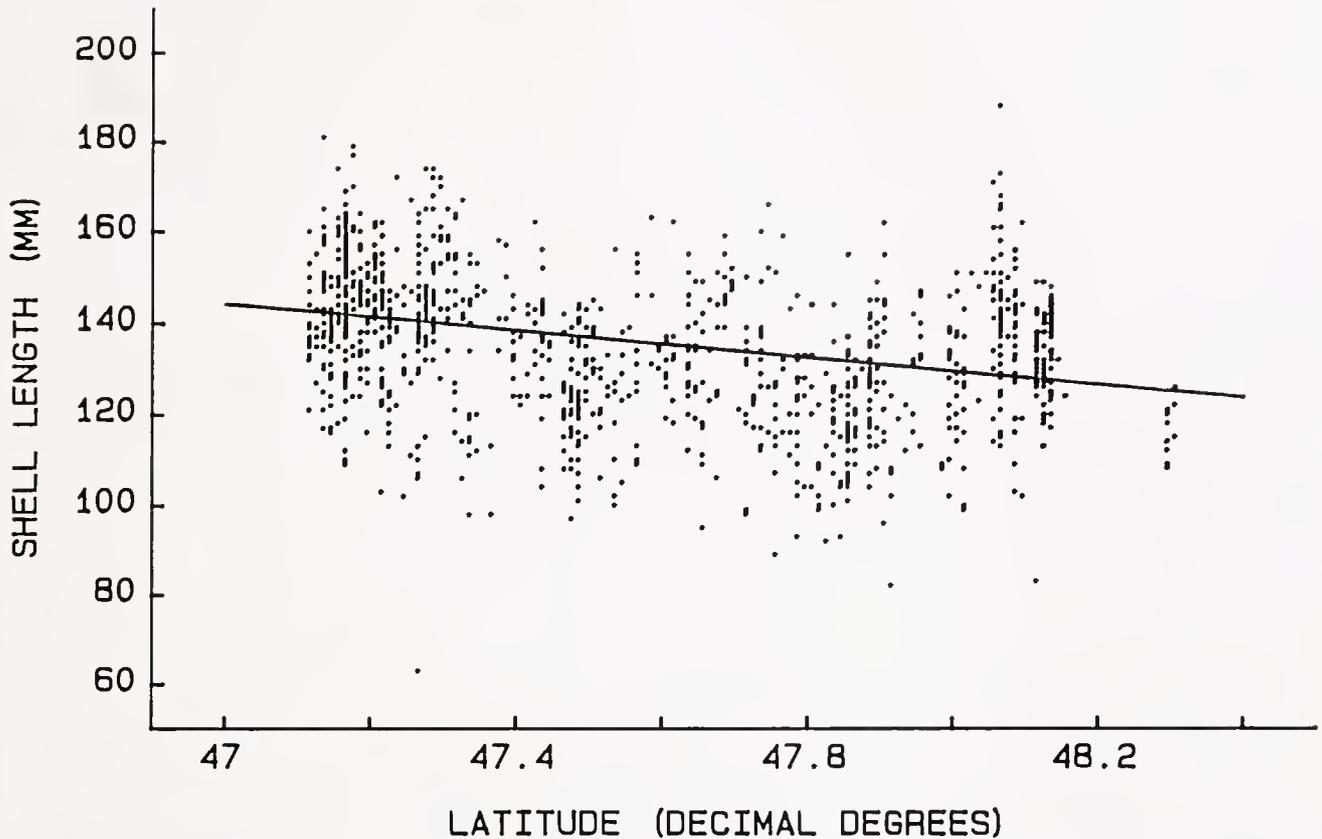


Figure 4. Mean shell length versus latitude in 1304 samples collected from Puget Sound between 1973 and 1985. Regression line, shell length =  $2860.20 - 705.41 \times \text{Ln}(\text{latitude})$ .

relationship (Fig. 5). The best fit line (log linear) flattens out with increasing water depth so geoducks from depths greater than 18 m would probably not show additional significant decreases in shell length. Geoducks were not dug in water deeper than 20 m so this prediction was not tested.

All tests for length discussed above were also done on natural log-transformed weight data. Similar results were obtained from the ANOVA ( $F = 97.6$ ,  $P < 0.001$ ) and regression analysis ( $F = 241.5$ ,  $P < 0.001$ ) except that the best fit from the regression analysis was a simple linear model.

We can conclude that geoduck length and weight decrease significantly as the water depth increases between 3 and 20 m.

#### Length and Weight Versus Sediment Type

The length and weight data were placed into four groups depending on the major component of the surface substrate layer as subjectively assessed by divers. For example, if the divers judged that the major component of the substrate surface sediment along a transect was sand and a geoduck sample was dug somewhere along this transect, then the length and weight data would be placed in the sand category.

The average shell length and whole wet weight (non-transformed) of geoducks in samples from the various sediments range from 128.8 mm and 835.2 g in pea gravel-gravel; to 130.2 mm and 783.5 g in mud; to 137.0 mm and 895.6 g in mud-sand; and to 138.6 mm and 907.2 g in sand.

The differences in the mean lengths and weights between the four substrate categories were assessed with a one-way ANOVA and the Newman-Keuls multiple range test. The mean lengths are significantly different in the four sediment types (ANOVA  $F = 7.9$ ,  $P > 0.001$ ). The Newman-Keuls test showed that there are only two size groups based on the four sediment categories. The mean length of geoducks in sand was not significantly different from the mean length of geoducks in mud-sand mixtures. The mean length of geoducks in mud was not significantly different from the mean length in pea gravel-gravel mixtures. There was a significant difference between the mean length of geoducks in sand and mud-sand sediments and the length of geoducks in mud and pea gravel-gravel sediments. The ANOVA for weight in the various substrate types showed no significant differences ( $F = 2.6$ ,  $P > 0.05$ ).

The lack of significant differences in weight is probably

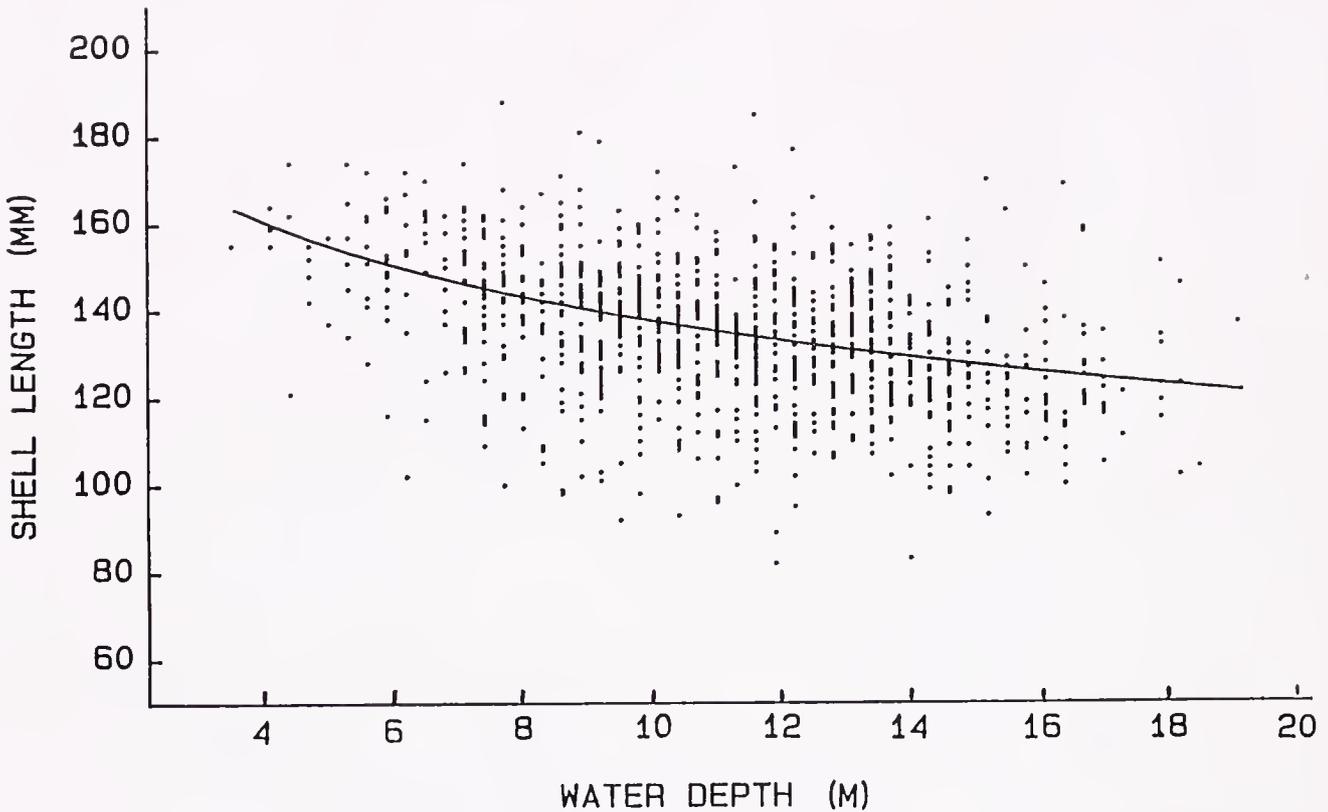


Figure 5. Mean shell length versus mean water depth in 1114 samples collected from Puget Sound between 1973 and 1985. Regression line, shell length =  $226.62 - 25.37 \times \ln(\text{water depth})$ .

due to the factors that affect the weight (drain time, injury by the water jet or variable shell thickness). All these factors increase the variability of the weight measurements compared to the length measurements, thereby making real differences in weight more difficult to detect.

#### *Geoduck Density*

The density frequency distribution of geoducks/m<sup>2</sup> (Fig. 6) shows that the data are definitely not normally distributed. The Taylor transformation produced a distribution that is closest to being normal (Fig. 7). If the two ends of the curve are trimmed off, i.e., below 1.19 and above 4.19, the resulting distribution is very close to normal. With the large data set (8589 quadrats) and the close approximation to a normal curve, we decided to use parametric tests on the transformed and trimmed data.

The average of 8589 untransformed, untrimmed, 41.8 m<sup>2</sup> quadrat counts was 69.3 geoducks (1.7 geoducks/m<sup>2</sup>; range was 0–22.5 geoducks/m<sup>2</sup>) with a standard deviation of 87.1 geoducks. This includes samples where no geoducks were found. The average density with zeros removed was 2.1 geoducks/m<sup>2</sup>.

The distribution in Figure 6 is a negative binomial, which means that geoducks are clumped or contagiously distributed. The average number of individuals per clump

is 109 and the average number of clumps per quadrat (Elliott 1971) is 0.64.

#### *Density Versus Geographic Area*

The densities of geoducks (non-transformed data) were 2.0, 1.9, 1.7, 0.6, and 0.2/m<sup>2</sup> in South Sound, Hood Canal, Central Sound, Strait of Juan de Fuca, and North Sound, respectively.

A one-way ANOVA of transformed and trimmed density data demonstrated a significant difference between geographic areas ( $F = 529.2, P < .001$ ). The Newman-Keuls test of the same data gave significant differences between all areas tested except Hood Canal and Central Sound.

#### *Density Versus Latitude*

A regression analysis was completed to discover if geoduck density is related to latitude (Fig. 8). The regression analysis revealed a significant inverse linear relationship ( $F = 341, P < 0.001$ ). Geoduck density decreases significantly along a south to north gradient.

#### *Density Versus Water Depth*

Geoduck density was examined in three different depth categories (<9.1 m, 9.1 m to 13.7 m, and >13.7 m). The

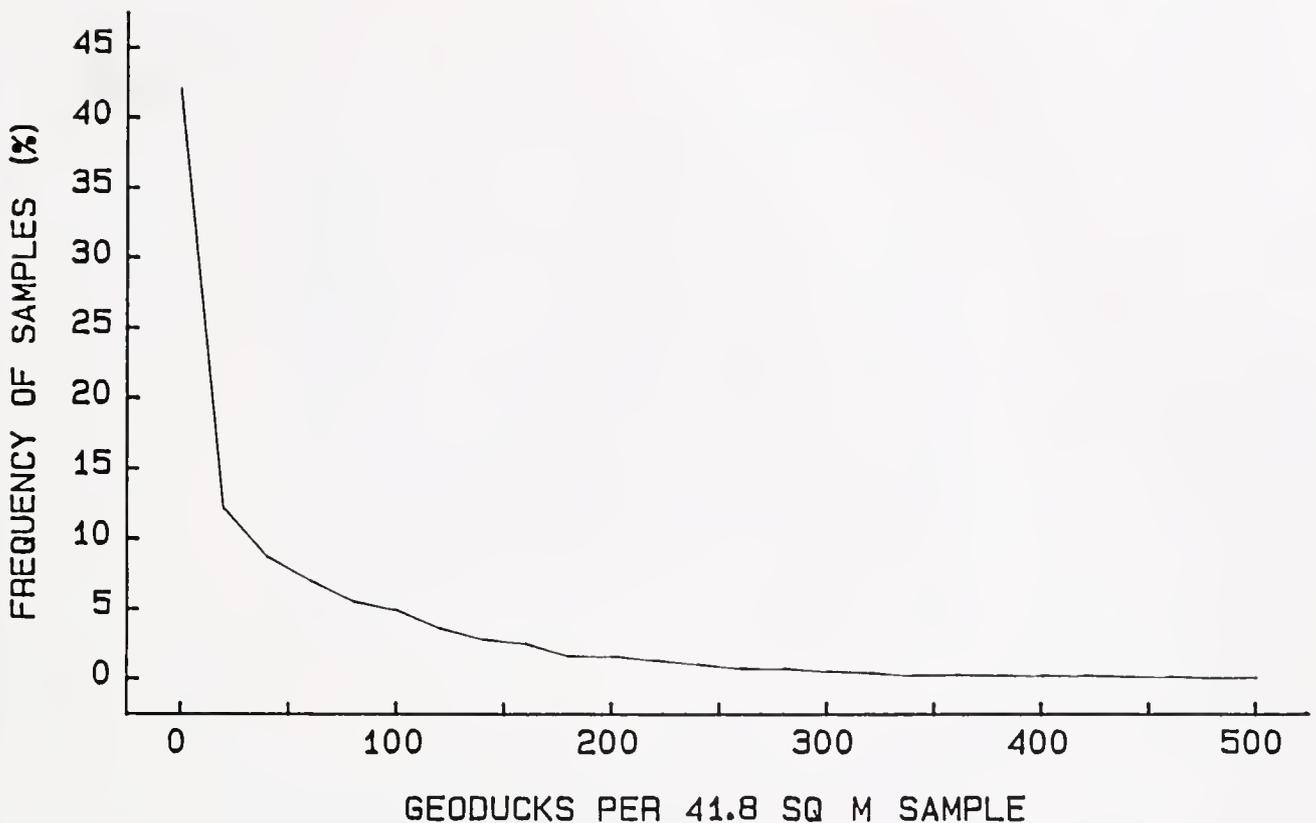


Figure 6. Frequency distribution of geoduck density in 8589 quadrats collected from Puget Sound between 1973 and 1985 including those with no geoducks.

lowest average density of nontransformed data was found in the shallow depth category (1.4 geoducks/m<sup>2</sup>). The middle depth category averaged 2.0 geoducks/m<sup>2</sup>, and the highest density (2.2 geoducks/m<sup>2</sup>) was found in the deepest depth.

A one-way ANOVA of transformed and trimmed data showed a significant difference in the density between the three categories ( $F = 64.8$ ,  $P < 0.001$ ). A Newman-Keuls test showed significant differences between each water depth category.

A regression analysis of Taylor-transformed and trimmed density data versus water depth showed a significant log-linear relationship ( $F = 223$ ,  $P < 0.05$ , Fig. 9). For this analysis the water depth of each quadrat was the median depth between the start and end of each transect. Geoduck density is positively correlated with water depth between 0 and 25 meters.

#### Density Versus Sediment Type

The geoduck density in numbers/m<sup>2</sup> (non-transformed) in the sediment types discussed earlier was 1.2 in mud, 2.0 in mud-sand, 2.1 in sand, and 1.8 in pea gravel-gravel.

A one-way ANOVA (transformed and trimmed data) showed that geoduck density was significantly different in the various substrate types ( $F = 53.6$ ,  $P < 0.001$ ). A

Newman-Keuls multiple range test showed significant differences in all combinations tested except between mud-sand and sand.

Geoducks were most dense in mud-sand or sand substrates. Another way to examine the relationship between geoducks and substrate types is to compare the percentage of samples taken in various substrate categories that have geoducks present (Table 1).

High percentages of the samples taken in mud-sand or sand categories contained geoducks (overall average were 92 and 88 for mud-sand and sand respectively), while only 47% and 61% of the samples taken in mud and peagravel-gravel categories contain geoducks.

#### Density Versus Length and Weight

Linear regression analyses were conducted to test for a relationship between geoduck density (transformed and trimmed) data and geoduck length and weight, but no significant relationships were found (Length  $F = 0.07$ ,  $P > 0.25$ ; Weight  $F = 0.35$ ,  $P > 0.25$ ).

#### Geoducks and Associated Flora and Fauna

##### Flora and Fauna in Geoduck Beds

The plants and animals commonly observed in geoduck beds are listed in Table 2. The organisms are arranged in

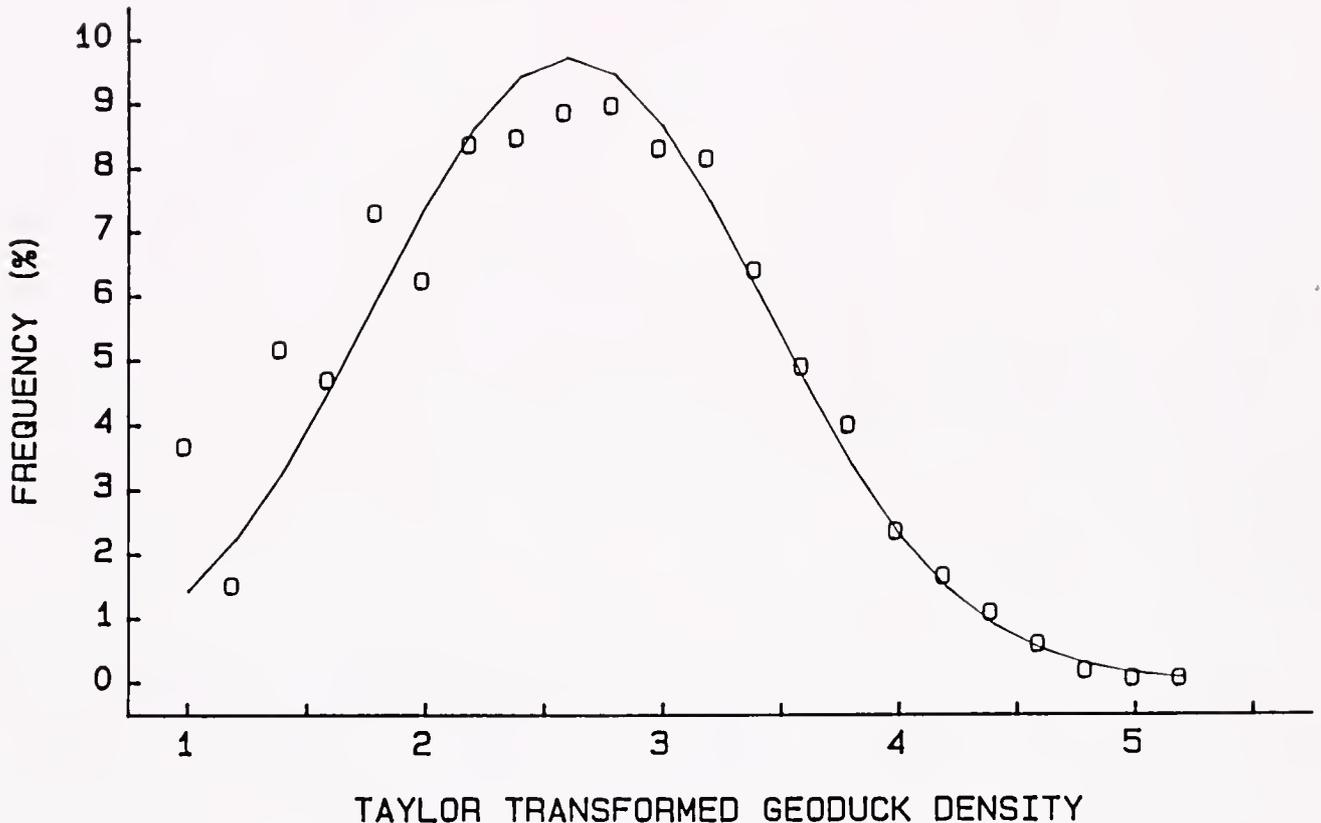


Figure 7. Expected normal distribution and the observed frequency after a Taylor transformation (based on geoducks per 41.8 m<sup>2</sup> quadrats). 0 = observed density and line = expected normal distribution.

descending order of the number of samples in which the particular plant or animal was observed. The organisms listed have been identified to the species level with ones that are easily caught and identified, but others are listed by major taxon.

Sixty-eight species or groups were observed, but only the 12 taxa occurring in 100 samples or more are shown in Table 2.

#### Flora and Fauna Versus Geoduck Density

Plants and animals that occurred in 100 samples or more were examined further to see if geoduck density in samples with these organisms is significantly different than in samples without the organisms (Table 3). Because geoduck density is not normally distributed and the associated species are probably not normally distributed, the Mann-Whitney nonparametric test was used in the analyses. These tests showed that geoduck density was significantly correlated with chaetopterid polychaetes, sea pens, horse clams, red rock crabs, moon snails, and kelp.

#### Geoduck Quality

##### Geoduck Quality Frequency Distribution and Tests for Normality

A geoduck quality index was calculated by the following formula: quality index =  $\text{Log} [(N1 \times 1000) + N2 + (N3$

$\times - 1000) + 100,000] \times 1000$  where log = natural logarithm, N1 = number of grade 1 geoducks, N2 = number of grade 2 geoducks, and N3 = number of grade 3 geoducks. The histogram of all quality data showed that the data was not normally distributed and could not easily be normalized with transformations. All statistical tests used on quality data were non-parametric.

#### Quality Versus Water Depth

The quality data from samples taken in the three previously used water depth categories (<9.1 m, 9.1 to 13.7 m, >13.7 m) were analyzed with a Kruskal-Wallis test (Zar 1974) and the quality was found to be significantly different in the three depth ranges tested (Chi-square = 5.99,  $P < 0.005$ ).

A Spearman correlation coefficient test was run on the quality and water depth data and a significant positive correlation ( $r_s = 0.119$ ,  $P < 0.002$ ) was found. As the water depth increases, the quality of geoducks measured as meat color generally decreases.

An ANOVA conducted between mean water depth of geoduck samples and four substrate categories discussed earlier showed a significant difference in water depths between the categories ( $F = 3.12$ ,  $P < 0.001$ ). A Newman-Keuls multiple range test showed that the average water depth of mud substrates was 12.2 m and was significantly

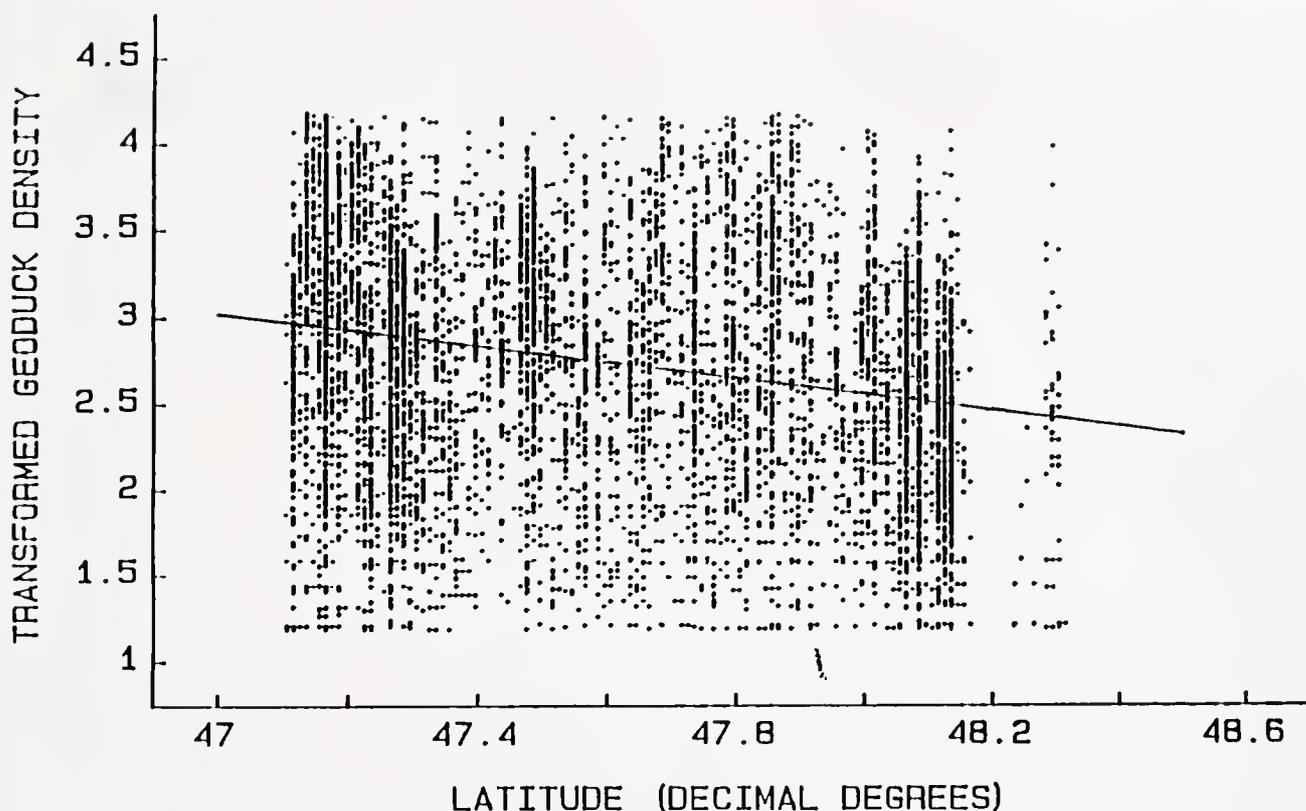


Figure 8. Geoduck density (Taylor-transformed and trimmed density based on 41.8 m<sup>2</sup> quadrats) versus latitude in 6131 samples collected in Puget Sound between 1969 and 1985. Regression line, geoduck density = 25.3 - 0.47 × latitude.

deeper than the depths of the other three substrate categories.

#### Quality Versus Substrate Type

The quality of geoducks in the four previously discussed substrate types was tested with a Kruskal-Wallis test (Zar, 1974). No significant differences in quality were found at the 95% probability level, but differences were present at the 90% probability level. The robustness of the non-parametric test was insufficient to detect differences at the 95% level. Unfortunately, there are no multiple range tests for the Kruskal-Wallis test to determine where the differences occur or, in this case, in which substrate the highest quality was found. The averages of quality index in the four substrate categories are given in Table 4. The highest quality index was found in mud-sand, sand, and peagravel-gravel and the lowest, in mud.

## DISCUSSION

#### *Geoduck Length and Weight*

Many factors can affect the size of geoducks found in any particular bed, including the productivity of the water, the water depth and current flow, the substrate composition, and the geographic area.

The largest geoducks in Puget Sound are found in South

Sound even though primary productivity is less here than in Central Sound (main basin, Strickland, 1983). Central Sound is very high in primary production on a yearly basis; however, the production comes in large blooms and is concentrated during the period April through August (86% of the yearly 462 g of carbon fixed per m<sup>2</sup>). Production in the South Sound, even though less than Central Sound, is spread out over a longer time period and is not so heavily concentrated in dense, short-lived blooms. The average summer water temperature (July through August, 0 to 20 meters) of the South Sound region is normally 2 to 3°C higher than the main basin (Collias et al.). The South Sound region, with warmer summer water and more even plankton production may be more conducive to overall growth of suspension feeders such as geoducks.

The inverse relationship of size with latitude is apparent only in Puget Sound. Geoducks in British Columbia are larger than those of Puget Sound even though the average latitude of geoduck beds in British Columbia is much higher than most of Puget Sound. Geoducks from five locations in the Strait of Georgia and the west side of Vancouver Island range from 134.0 to 163.9 mm of shell length (Breen and Shields 1983). In another study 1774 geoducks were obtained from commercial processors. The clams had been taken from 10 different locations in British Columbia. The average length was 150 mm and the range was 90 to

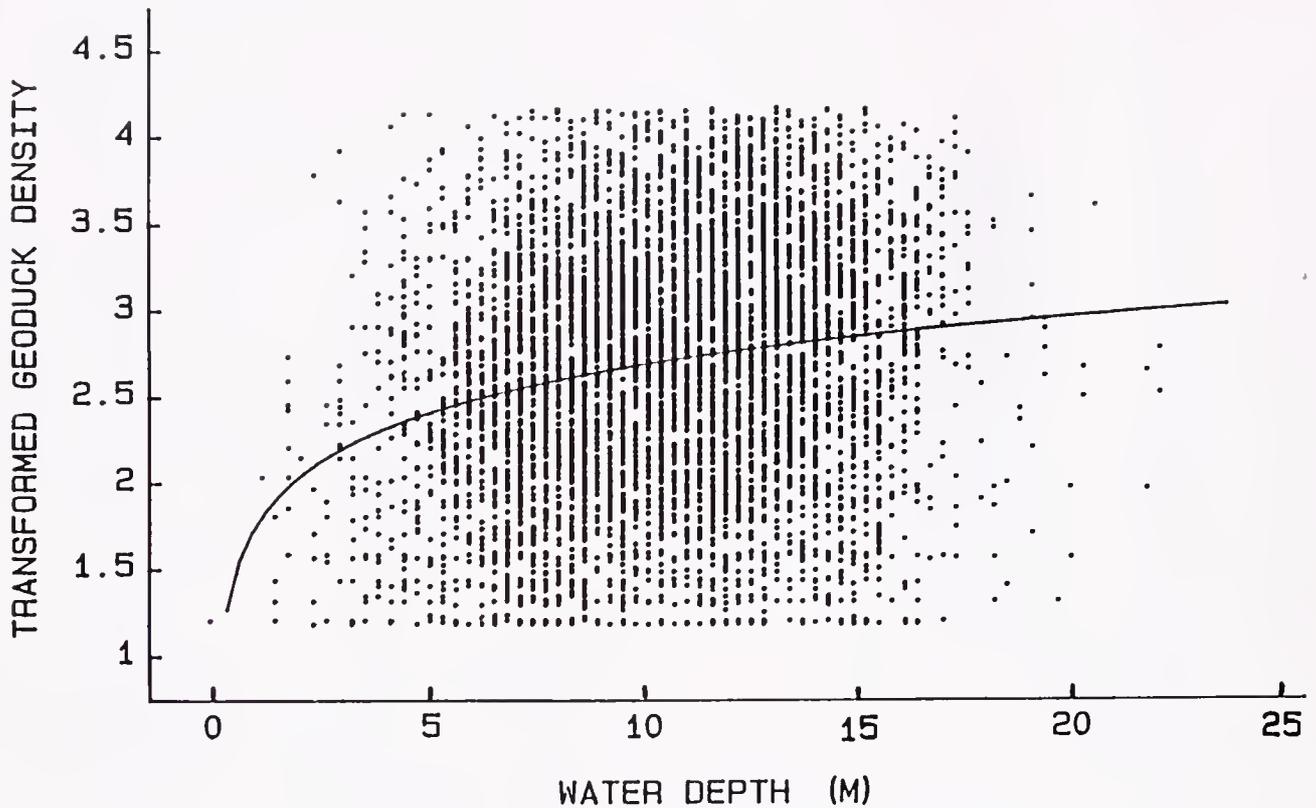


Figure 9. Geoduck density (Taylor-transformed and trimmed data) versus mean water depth in 6117 samples collected from Puget Sound between 1969 and 1985. Regression line, geoduck density =  $1.27 + 0.40 \times \ln(\text{water depth})$ .

195 mm (Harbo et al. 1983). Although geoducks are larger in British Columbia than in Puget Sound, the largest individual measured was found in Puget Sound (212 mm shell length and 3250 g whole wet weight).

Geoducks which grow in compact sediment such as pea gravel-gravel or shell substrates are often small and misshapen from being confined among the relatively large, hard materials. The substrate itself can reduce the size to which clams would grow if they were in less compact material. Clams occurring on top of layers in the substrate which they cannot penetrate to assume their normal burial depth

usually have short siphons and small size (WDF unpublished data), and their susceptibility to digging predators such as sea stars is increased (Sloan and Robinson 1983).

Geoducks taken from mud were significantly smaller in shell length than those in mud-sand or sand. This may be due to factors associated with the water current speed and not the nature of the substrate. Low current areas are deposition zones of fine sediments. Clams growing in these low current areas probably receive less food than those of high current areas and thus grow to a smaller size.

#### *Geoduck Density*

Geoducks in this study were found to be contagiously distributed. Fyfe (1984), in a study done on the west coast of Vancouver Island, British Columbia, also found that geoducks were clumped but that the numbers per clump were much lower than in Puget Sound. Within the clumps geoducks are either random or clumped. Andersen (1971) found in intertidal plots in Hood Canal clumped and random distribution in 30.5 m  $\times$  30.5 m plots. In a study in 9.1 m of water in Hood Canal, juvenile geoducks were found clustered around adults (Goodwin and Shaul 1984). Fyfe (1984) also found that the nearest neighbors of juvenile geoducks are usually full grown adults. The clumping of juveniles around adults may result from the attraction of setting larvae to adults (pheromones) or perhaps the in-

TABLE 1.

Percentage of transects with geoducks present in six regions of Puget Sound.

Region	Percentage of samples with geoducks				All
	Mud	Mud-sand	Sand	Peagravel-gravel	
South Sound	64	94	90	57	82
Central Sound	72	95	90	67	86
Hood Canal	76	89	95	63	83
Strait of Juan de Fuca	38	86	79	58	69
North Sound	0	61	51	25	45
San Juan Islands	0	0	0	0	0
All areas	47	92	88	61	81

TABLE 2.

Occurrence of plants and animals in quadrats taken between 1982 and 1985.

Plant or animal	Number of quadrats with species or group
Chaetopterid polychaetes	
<i>Sphiochaetopterus costarum</i>	
<i>Phyllochaetopterus prolifica</i>	529
Sea Cucumber	
<i>Parastichopus californicus</i>	429
Sea pen	
<i>Ptilosarcus gurneyi</i>	321
Kelp	
<i>Laminaria</i> sp.	257
Horse clams	
<i>Tresus</i> sp.	215
Large red algae	
Phylum Rhodophyta	177
Small red algae	
Phylum Rhodophyta	162
Sunflower star	
<i>Pycnopodia helianthoides</i>	124
Red rock crab	
<i>Cancer productus</i>	123
Short spined starfish	
<i>Pisaster brevispinus</i>	118
Moonsnail (and/or egg cases)	
<i>Polinices lewisii</i>	114
Ample panomya	
<i>Panomya ampla</i>	103

crease in juvenile survival next to adults. No relationship was found between geoduck density and size. Fyfe (1984) also found this to be true in British Columbia.

The contagious distribution may be due to the patchy distribution of preferred substrates, attraction of adult conspecifics for settled larvae, or the presence or absence of other plants or animals. Cooper and Pease (1987) have shown that tubes of two chaetopterid polychaetes will elicit a significant metamorphosis response in larval geoducks. The density of geoducks is positively correlated with the presence of these polychaete worm tubes. Differential setting and/or survival in the patches of worm tubes could explain the patchiness in adult geoducks. Other positive correlations are probably coincidental, i.e., geoducks and the other organisms co-occur simply because they both prefer the same habitat with little or no biological interaction. The association of starfish, crabs, soles, flounders, and moon snails with geoducks could be due to attraction of these animals to geoducks as a food source because all are known to be predators of geoducks.

The average geoduck density of our transects was 1.7 clams/m<sup>2</sup>. The range in density was 0 to 22.5 geoducks/m<sup>2</sup>. The average weight of these geoducks was 872.2 g. Therefore, the average standing stock was 1483 g/m<sup>2</sup>. The highest standing stock was 19,650.7 g/m<sup>2</sup>. Very frequently geoducks dominate the benthic biomass (weight per unit

TABLE 3.

List of species where geoduck density was significantly different than in samples without those species (non-transformed or trimmed data).

Organism		Geoduck density (number/m <sup>2</sup> )	Mean water depth
Chaetopterid polychaetes	(1)***	2.1	39
Sea pen	(1)***	2.6	39
Horse clam	(1)***	2.3	35
Large red algae	(2)***	0.4	33
Small red algae	(2)**	1.3	33
Red rock crab	(1)**	2.4	35
Moon snail and/or egg cases	(1)*	2.3	38
Laminarian kelp	(1)*	1.0	33

(1) = geoduck density significantly higher in quadrats where species was present

(2) = geoduck density significantly lower in quadrats where species was present

\* = P < 0.05

\*\* = P < 0.01

\*\*\* = P < 0.001

area) (Goodwin 1978). The overall range in the density data is quite wide. The range in density from individual beds (3.2 to 51.8 hectares) which have been leased for commercial fishing in Puget Sound in the last few years is much narrower—0.3 to 4.9 clams/m<sup>2</sup> ( $\bar{x}$  = 2.1/m<sup>2</sup>). Breen and Shields (1983) reported density figures from five geoduck beds in British Columbia which ranged from 0.2 to 14.7 geoducks/m<sup>2</sup>. Four of the five beds averaged less than 2 geoducks/m<sup>2</sup>. Fyfe (1984) reported densities of 0 to 13/m<sup>2</sup> with a mean of 4.9 geoducks/m<sup>2</sup> in 1 m<sup>2</sup> quadrats from a bay on the west side of Vancouver Island, British Columbia. Harbo and Peacock (1983) stated that aggregations as high as 38 geoducks/m<sup>2</sup> have been found in British Columbia; however, they did not mention the size of the samples from which these figures were derived. Geoducks may be that dense in Puget Sound over very small areas, but not in areas as large as our 41.8 m<sup>2</sup> quadrats. The limited data from Canada suggests that densities are higher than in Puget Sound. Thus, the inverse relationship between geoduck density and latitude does not extend north into British Columbia.

Geoducks are found in Puget Sound from the lower intertidal zone (Andersen 1971) to water depths of at least

TABLE 4.

Geoduck quality index versus sediment type.

	Mud	Mud-sand	Sand	Peagravel-gravel
x	11,550	11,832	11,737	11,785
SD	418	335	525	302
N	10	170	43	56

TABLE 5.

Geoducks observed by underwater T.V. in Case Inlet.

Water depth (m)	Average geoduck siphon counts
6-12	3.2
13-18	3.5
19-24	15.5
25-30	6.0
31-36	4.2
37-42	3.3
43-48	2.3
49-55	1.3
56-61	2.0

110 m (Jamison et al. 1984). The majority of the samples in this study were taken between 6.1 and 18.3 m of water. State law prevents commercial geoduck fishing shallower than 5.5 m, and diving below 18.3 m greatly restricts the time a diver can dive on a no-decompression basis. Therefore, very few samples were taken in water less than 5.5 m or deeper than 18.3 m. Even though we have very few samples in water less than 5.5 m, our divers in the past 20 years have spent considerable time making observations in these areas and we can state with confidence that the decline of geoduck density with decreasing water depth (Figure 9) continues to the upper limits of their vertical distribution.

From our own data we cannot determine the distribution of geoduck density at water depths greater than about 20 m. However, work done by Washington State Department of Natural Resources (Jamison 1984) indicates that in one bay in South Sound (Case Inlet) geoduck density increases down to 24 m, then decreases below that point (Table 5). The data in Table 5 are counts of geoduck siphons or shows appearing on the T.V. monitor per unit of time. Only T.V. transects that were completed at right angles to the shoreline were used in the analysis. A regression analysis of our diver transects from Case Inlet (6 to 18 m) showed a significant direct relationship between water depth and geoduck density ( $F = 28$ ,  $<0.001$ ,  $n = 440$ ). In other underwater T.V. work completed by WDF (unpublished data) geoducks were found in two bays in South Sound in water over 46 m deep.

From this information we can conclude that significant

numbers of geoducks exist in water of up to 61 m in one bay in Puget Sound and probably do so in others.

Standing stock estimates were calculated from the three water depth categories previously discussed ( $<9.1$  m, 9.1 m to 13.7 m, and  $>13.7$  m). The estimates were obtained by multiplying the average geoduck weight and density for each depth category. The estimates are 1510.3, 1750.0, and 1557.0 g/m<sup>2</sup> for the water depth categories ( $<9.1$  m, 9.1 m to 13.7 m, and  $>13.7$  m) respectively. The estimates suggest that biomass increases from the shallow water depths to the intermediate depths, then decreases again below this water level.

#### Geoduck Quality

Geoduck quality is inversely correlated with water depth. The reason for this relationship is unclear. Age of geoducks is negatively correlated with quality (personal observation). The younger the clam, the more likely that the meat color will be #1. Unfortunately we have no age data in relation to water depth to determine if clams living in deeper water are older than those in shallow water. This situation could occur if mortality rates were higher in shallow water compared to deeper water. Geoducks are more numerous in deeper water compared to shallow water; however, we don't know whether this is due to differential mortality rates or not. Another explanation for the relationship between water depth and geoduck quality may be the fact that in Puget Sound as the water depth increases it is more likely that the substrate will be composed of mud instead of sand or pea-gravel-gravel, and geoduck quality is related to substrate type.

Age and shell length are not well correlated (Fyfe 1984). Geoducks are very long-lived animals; however, most of the growth in shell length occurs during the first ten years of life. Shell thickness, however, increases throughout life (Shaul and Goodwin 1982, Goodwin and Shaul 1984, Sloan and Robinson 1984). Age and quality are related; therefore, quality and shell length are not expected to be strongly correlated.

After digging many thousands of geoducks from hundreds of locations throughout Puget Sound, we believe that the coarser sand and pea gravel-gravel produces higher quality geoducks compared with mud substrates, particularly those with high organic content.

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## SEASONAL CONDITION CHANGE, MORPHOMETRICS, GROWTH AND SEX RATIO OF THE OCEAN QUAHOG, *ARCTICA ISLANDICA* (LINNEAUS, 1767) OFF NEW JERSEY, U.S.A.

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**ABSTRACT** There was little seasonal trend in any condition index calculated for specimens of *Arctica islandica* collected off New Jersey monthly from November 1988 through October 1989. Tissue weights calculated for standard-sized (95 mm shell length) individuals each month also had no clear seasonal variation. Total calculated monthly wet tissue weights varied from 37.9 to 51.3 g, or 15% below and above the annual mean (44 g). Total calculated monthly dry tissue weights varied from 8.4 to 13.6 g, or approximately 25% below and above the mean (10.7 g). Almost all variation in computed dry tissue weight between months was due to variation in weight of the viscera (which included the gonad), while there was little month-to-month change in somatic tissue weight. It is suggested that site-specific differences in growth rate and reproductive cycling, and/or lack of synchrony of reproductive cycles of individuals at a given site contributed to the observed annual index pattern.

Males had greater mean condition index values (based on total dry tissue weight divided by internal shell volume) in spring and summer, and higher visceral index values (visceral dry tissue weight divided by total dry tissue weight) throughout the entire year than females. Males ( $n = 168$ ) were outnumbered by females ( $n = 218$ ) 44% to 56%, yielding a male:female ratio of 0.77:1; no hermaphrodites were found. Mean shell length of females was larger than for males each month. A canonical discriminant function based on shell height and the natural log of shell weight estimated sex correctly in 257 of 386 individuals (67%), or only slightly better than chance (50%). Results of growth line analyses of a sub-sample of quahogs suggest that females may live longer and have a larger asymptotic size than males.

**KEY WORDS:** *Arctica islandica*, ocean quahogs, condition, shell morphometrics, growth, sex ratio

### INTRODUCTION

The ocean quahog, *Arctica islandica* L. is harvested in large quantities off the east coast of the United States. In 1989, U.S. landings were approximately 23,000 metric tons of meat with an estimated dock-side value exceeding \$16 million (NOAA 1990). Little is known of seasonal changes in condition (the ratio of tissue weight to shell weight or volume) in the mid-Atlantic Bight, where the fishery for the species is concentrated (NOAA 1988). Ropes (1971) determined shell length-meat weight relationships for quahogs collected at two locations in the mid-Atlantic Bight in June. Murawski and Serchuk (1979) suggested a latitudinal gradient in shell length-meat weight in samples obtained in February–March, in which size-specific meat weight increased with decreasing latitude. In their samples, however, individuals at different latitudes may have been at different stages in their reproductive development which could have affected their conclusions. Murawski et al. (1982) reported that size-specific meat weights of quahogs from a single location were lower in August 1979 than in February 1980, but differences in meat weight were small and only two samples were used in the study. The present study was initiated to examine monthly changes in condition of *A. islandica* off New Jersey using samples obtained from a commercial processor. Another objective of the study was to determine the similarity be-

tween female and male ocean quahogs in their shell morphology and seasonal condition cycles.

### MATERIALS AND METHODS

Samples of ocean quahogs were obtained once each month from November 1988 through October 1989 from a clam processor in Wildwood, NJ. A sample consisted of approximately 40 individuals selected from the catch of a single vessel. Catch location was available for only four of the twelve samples. Two samples (November 1988 and June 1989) were collected approximately 56 km SE of the mouth of Cape May Harbor (38°35.05'N, 74°22.68'W; 38°34.45'N, 74°20.35'W, respectively). Another two samples (May and September 1989) were collected farther north and east, at approximately 80 km ESE and 61 km E, respectively, of the mouth of Cape May Harbor (38°43.04'N, 73°50.92'W; 38°50.51'N, 74°03.56'W, respectively). Depth at each of the known collection sites was between 40–45 m. Based on interviews with personnel at the processing plant, there was no reason to suspect that the other eight samples were collected from areas far-removed from those listed above.

Quahogs were transported to the lab on ice where they were sacrificed within 24–48 h. Time intervals from when the clams were caught until they were collected from the plant ranged from 4–24 h. Several shell morphometric and tissue weight measurements were obtained (Table 1). Soft tissues were removed and dissected to measure drained wet and dried (at 60–70°C for between 6–8 d) somatic, vis-

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ceral and total tissue weights to the nearest 0.01 g on a top-loading balance. Dry tissue weights of between 28–30 individuals were measured each month (total N = 357). Sex of either 30 or 40 individuals each month was determined by gonad biopsy (total N = 386). A pipette was inserted in the gonad through the body wall and a small quantity of gonadal tissue was withdrawn, placed on a microscope slide and examined at 100× for the presence of eggs or sperm.

Three indices of condition were calculated for each individual specimen (Table 1). Means of the individual arcsin  $\sqrt{p}$  transformed condition and visceral index values ( $\pm$  standard error) were calculated separately for females, males and the total sample each month (Sokal and Rohlf 1981). Reported condition and visceral index values are back-transformed to percent. Separate least-squares linear regressions were calculated for females, males and the total sample each month relating shell length and SDW, VDW, TDW and TWW (Table 1). In each case, the natural log of the tissue weight was regressed against the untransformed shell length, yielding an equation which was used to calculate the tissue weight ( $\pm$  95% confidence intervals) of a standard-sized (95 mm SL, approximate mean SL of all specimens; Table 1) specimen. Various linear regressions comparing morphometric and tissue measurements were calculated to distinguish any statistical morphometric differences between females and males. Step-wise and canonical discriminant analyses (SAS 1988) using the five shell morphometric variables and one tissue weight variable (VDW) were used to generate a function to discriminate between males and females using shell morphometry and visceral tissue weight. Step-wise procedures were used to determine which morphometric parameters were most powerful in discriminating between the sexes. Canonical discriminant analysis using two classes (sexes) is a dimension-reduction technique in which a new variable is com-

puted which should be negative for one class, in this case males, and positive for the other.

Growth line analyses were conducted using the left valves of 22 females and 25 males collected in May and June 1989. Methods of embedding, polishing and acetate peel preparation were those of Ropes (1985). Prismatic sublayers within the inner complex-crossed lamellar layer and/or the cardinal tooth were counted on a single specimen (at 40 and 100× on a compound microscope; Ropes et al. 1984a) until three counts (by the same reader) were within 10% of one another. Best-fit equations relating SL and the mean of the three growth line counts were generated separately for females and males. Data for females had a strong linear component and an equation was fit using least-squares linear regression. Data for males had a strong curvilinear component and a nonlinear regression model of the form,

$$SL = B + Ce^{K(GL)},$$

(where SL = shell length, B = an estimate of asymptotic shell length, e = the base of the natural logarithm, GL = number of growth lines, and C and K = constants) was used. Loss functions were computed using a SIMPLEX procedure (Wilkinson 1988). Starting values of 100, -10 and -0.01 were used for estimating the coefficients B, C and K, respectively. Loss functions were minimized within 13 iterations. Several unsuccessful attempts were made to fit the female data to this model; after 30 or more iterations, estimates of coefficients had not converged.

## RESULTS

### *Condition by Month and Season*

Plots of CSV and VI (based on dry weight) by month revealed no clear seasonal trend for either females or males (Fig. 1A & B). However, the patterns of monthly CSV and

TABLE 1.

Shell morphometric and tissue weight measurements, and condition indices calculated for each specimen of *Arctica islandica*. Abbreviations for each measurement and index used in the text are in parentheses.

### A. Shell Morphometric Measurements

1. Length (SL): greatest antero-posterior distance (to 0.1 mm)
2. Height (SH): greatest distance from umbo to ventral shell margin (not perpendicular to SL; to 0.1 mm)
3. Width (W): greatest distance through both valves (to 0.1 mm)
4. Volume (SV): total internal volume measured by filling each valve with water and measuring to the nearest 1 ml; if one of the two valves was broken, the volume of the intact valve was multiplied by two to obtain total volume
5. Weight (SW)—total air-dried (at least 24 h) weight of both valves measured to the nearest 0.1 g

### B. Tissue Weight Measurements

1. Somatic tissue wet (SWW) and dry weight (SDW)—includes mantle, gill, foot and adductor muscles
2. Visceral tissue wet (VWW) and dry weight (VDW)—all remaining tissue after soma removed, including gonad, digestive tract, etc.
3. Total wet (TWW) and dry weight (TDW)—summation of SWW and VWW, and SDW and VDW, respectively

### C. Condition Indices

1. Shell weight basis,  $CSW = [(TDW \text{ or } TWW)/SW] \times 100$
2. Shell volume basis,  $CSV = [(TDW \text{ or } TWW)/SV] \times 100$
3. Visceral index,  $VI = (VDW/TDW) \text{ or } (VWW/TWW) \times 100$

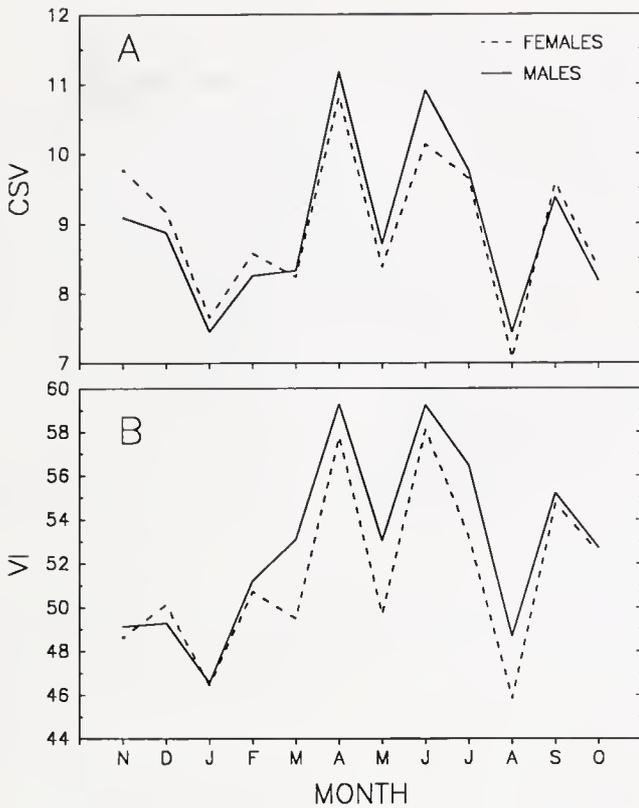


Figure 1. A. Monthly mean condition index (CSV = TDW/SV × 100) based on dry total tissue weight (TDW) and shell volume (SV); B. Monthly mean visceral index (VI = VDW/TDW × 100) based on dry visceral (VDW) and total tissue weights (TDW); indices are shown for both for male and female specimens of *Arctica islandica*.

VI values for females and males paralleled each other throughout the year. All condition indices (including CSW —not shown) exhibited the same pattern across the series of months, with declining values from November through January, increasing values through April, and considerable variation through the end of the sampled period in October. Highest values of CSW were recorded in April and July, and lowest values in August and October. Highest values of

CSV and VI were recorded in April and June, and lowest values in January and August (Fig. 1). Two-way analysis of variance (mixed model ANOVA) of all CSV and VI data on month and sex revealed that monthly means were significantly different from one another (Table 2), but no strong seasonal trends were apparent (Fig. 1). CSV appeared to be a better estimate of condition than CSW since correlation coefficients relating TDW and SL with SV were greater than those with SW for the entire sample (Table 3, 1–4).

Two-way ANOVA of all CSV data on month and sex revealed no statistical differences in CSV between the sexes across all months (Table 2). However, females had greater CSV than males from November through February, and from September through October, while males had greater CSV than females from March through August (Fig. 1A). Two-way ANOVA of all VI data on month and sex revealed that the sexes were significantly different from one another (Table 2), with males having greater values of VI than females in 11 of the 12 months sampled (Fig. 1B). Runs tests (Sokal and Rohlf 1981) on the distribution of female and male monthly indices with respect to each other revealed that both the CSV and VI patterns were significantly different from random. Results of these statistical tests suggest that: (1) males had greater CSV than females in spring and summer, while females had greater CSV than males in fall and winter; and (2) males had greater VI than females throughout the year.

Monthly mean shell length ranged from 81.9 mm in January 1989 to 99.3 mm in December 1988. The January sample was the only one with a mean shell length less than 90 mm. The total range in shell length for all samples was 72.5–120.9 mm. Condition based on shell volume (CSV, arcsin-transformed variable) was uncorrelated with both SL and SV (Table 3, 5–6). Thus, differences in size of the sampled animals had no effect on differences in monthly mean CSV (Fig. 1). Visceral index (VI, arcsin-transformed variable) was weakly correlated with both SL and SV (Table 3, 7–8), such that VI increased directly with both morphometric variables. Consequently, some of the de-

TABLE 2.

Results of two-way ANOVA's of condition index (based on shell volume, CSV) and visceral index (VI) on month and sex. SS = sum of squares, df = degrees of freedom, MS = mean square, F = F-ratio, p = probability; if p > 0.05, cannot reject null hypothesis that source of variation had no effect on differences in dependent variable.

Dependent Variable	Source of Variation	SS	df	MS	F	p
CSV	Month	380.12	11	34.56	29.88	p < 0.001
	Sex	<0.01	1	<0.01	<0.01	p > 0.99
	Month X Sex	12.30	11	1.12	0.97	p = 0.48
	Error	381.68	330	1.16		
VI	Month	1565.22	11	142.29	28.32	p < 0.001
	Sex	50.28	1	50.28	10.01	p = 0.002
	Month X Sex	58.44	11	5.31	1.06	p = 0.40
	Error	1657.91	330	5.02		

TABLE 3.

Correlation coefficients for each pair of morphometric, tissue weight or condition index parameters.  $r$  = correlation coefficient,  $N$  = number of data pairs,  $p$  = probability: if  $p > 0.05$ , cannot reject null hypothesis that parameters are uncorrelated. See Table 1 for key to parameter abbreviations.

	Parameters	$r$	$N$	$p$
1	SV and TDW	0.74	354	$p < 0.001$
2	SW and TDW	0.66	356	$p < 0.001$
3	ln SV and SL	0.94	383	$p < 0.001$
4	ln SW and SL	0.85	386	$p < 0.001$
5	SL and CSV	0.11	354	$p > 0.05$
6	SV and CSV	0.10	354	$p > 0.05$
7	SL and VI	0.20	356	$p < 0.01$
8	SV and VI	0.23	354	$p < 0.01$

crease in mean VI between December and January, for instance, could have resulted from the large decrease in mean SL between the two samples.

Calculated dry tissue weights for standard-sized specimens (SL = 95 mm) revealed no clear seasonal trends for any combination of tissue type (TDW, SDW and VDW) and sample (females and males; Fig. 2 (SDW not shown)). TDW was greatest in April, June and July and lowest in January and August. These were also months of relatively high and low CSV and VI, respectively. Monthly calculated TWW of a standard-sized specimen (pooling males and females) ranged from 37.9 to 51.3 g, or approximately 15% below and above the annual mean (44 g;  $\ln \text{TWW} = 0.0308(\text{SL}) + 0.8589$ ;  $n = 477$ ,  $r = 0.88$ ). Monthly calculated TDW for a standard-sized specimen (pooling males and females) ranged from 8.4 to 13.6 g, or approximately 25% below and above the annual mean (10.7 g;  $\ln \text{TDW} = 0.0320(\text{SL}) - 0.6803$ ;  $n = 357$ ,  $r = 0.83$ ). SDW exhibited the least variation of the tissue weights analyzed, with monthly values not exceeding 16% nor lower than 13% of the annual mean for males, females and both sexes pooled.

Month-to-month variation in VDW was greater than for SDW for both sexes. The highest values of VDW (April 1989) were between 38–49% greater, while the lowest values (August for females and January for males) were approximately 30% lower than the annual means for each sex individually. The monthly pattern of VDW was identical to that of TDW for both sexes (Fig. 2). Almost all of the variation in TDW (and CSV and VI as well) between months was due to variation in VDW.

#### Sex Ratio and Morphometric Differences between Males and Females

A total of 168 males (44%) and 218 females (56%) were identified in this study, yielding a male:female ratio of 0.77:1. This ratio was significantly different from 1:1 based on a chi-square test ( $\chi^2 = 6.477$ ;  $p < 0.025$ ). Females outnumbered males in all samples except May (16 males, 14 females). Only one specimen was of indeter-

minate sex, and no evidence of hermaphroditism or ambisexuality was observed.

Mean SL of females was greater than for males each month (by between 2.6–13.3 mm) and across all samples (mean SL: males = 91.1 mm, females = 95.9 mm; range in SL: males = 72.5–108 mm, females = 75.4–120.9 mm). When males and females from all samples were pooled by 5 mm length categories, it can be seen that although modal length categories were identical (90–95 mm; Fig. 3A), median SL was greater for females (95.6 mm) than males (91.2 mm; Fig. 3B). With increasing shell length, 5 mm categories were increasingly dominated by females. The percentage of males decreased from 47% of the specimens between 95.0–99.9 mm SL to 0% greater than 110 mm SL. Only 11% of all males, but 36% of all females had shell lengths greater than 100 mm. Of the 98 specimens with shell lengths greater than 100 mm, only 19 were males.

Results of covariance analyses of a series of regressions of morphometric parameters of males and females are given in Table 4. Of the thirteen tests of equality of slope

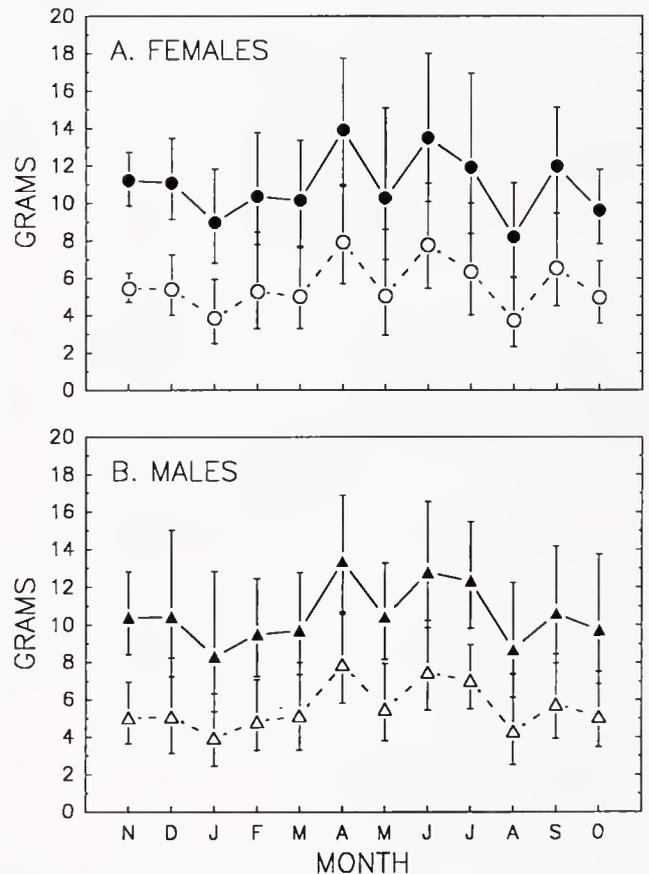
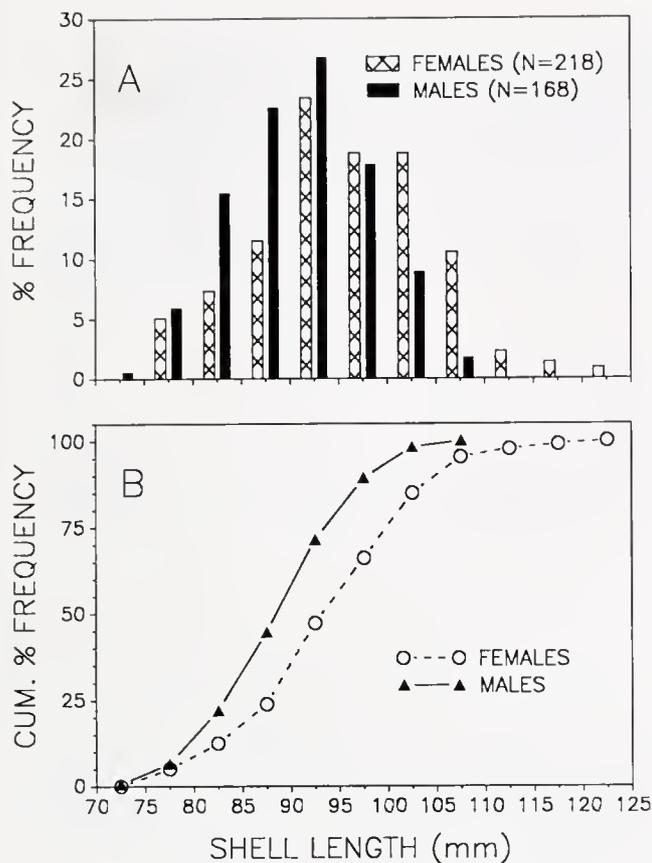


Figure 2. Total (solid symbols and lines) and visceral (open symbols and dashed lines) dry weights for a standard 95 mm shell length specimen of *Arctica islandica* ( $\pm 95\%$  confidence interval) calculated from separate least-squares linear regressions (natural log-transformed tissue weight against shell length) for each month and sample (A. Females; B. Males).



**Figure 3.** Percent length-frequency (A) and cumulative percent length-frequency (B) by 5 mm shell length category of male and female specimens of *Arctica islandica* collected throughout the course of the study. Bars and symbols are plotted at the mid-point of each shell length category.

(regression coefficient), only one, VDW vs. SV, yielded a significant difference between males and females; for a given VDW, males had significantly smaller SV than females. This is also revealed in the test of estimated Y for this pair of parameters. These results are supported by the significantly greater VI for males than females revealed in the two-way ANOVA and runs-test. Males also had smaller SV for given values of TWW and TDW over the course of the entire study, as well as lower SDW for given values of SL than females. Males had significantly greater values of VDW with respect to SL than females, which corresponds with the results with respect to SV. Thus, seasonal differences in CSV between males and females may have resulted from greater VDW in males than females (with respect to SL and SV) throughout the year, but especially in spring and summer. With respect to shell morphometry, males had significantly smaller height and greater weight for given lengths than females. The paired comparison which yielded the greatest difference between males and females was that relating shell height and weight, with males having significantly heavier shells than females (Table 4; Fig. 4; Y-intercept test,  $F = 24.37$ ,  $p < 0.001$ ).

Results of the series of regressions of morphometric parameters suggested that a function could be generated to discriminate between males and females based on shell morphometric characteristics. Using a canonical discriminant procedure (CANDISC, SAS 1988) and all five shell morphometric parameters (SL, SH and W; ln SW and ln SV), a discriminant variable, Y, was generated. When this function was applied to the data (Table 5), it was correct for 67.1% (257 of 383) of the sexed individuals on which all five shell morphometric characteristics were measured. Inclusion of VDW did not increase the sex-discriminating power of the generated canonical variable. Results of a step-wise discriminant procedure (STEPDISC, SAS 1988) suggested that most of the sex discriminating power of shell morphometry was contained in the parameters height and weight. The discriminant variable Z (Table 5; Fig. 5), based on only two variables, had 92% of the discriminating power of Y (ratio of squared distances between means) and was correct for 66.6% (257 of 386) of the sexed individuals. The distributions of Y and Z were similar to one another (that for Z is shown in Fig. 5), with approximately 70% of the male values negative and 65% of the female values positive. With an average discriminating power of approximately 67%, the probability of a correct sex determination using either Y or Z was only slightly greater than chance alone (50%).

Growth line analysis of a sub-sample of 22 female and 25 male quahogs suggests that female quahogs may have greater longevity, faster growth rates and a larger asymptotic size than males, if growth lines are deposited at the same periodicity by both sexes (Table 6; Fig. 6). The lack of a curvi-linear component to the female growth equation could have been due to the small number of females analyzed that had fewer than 60 growth lines and were smaller than 90 mm SL. Using the best-fit equations, shell lengths of females and males at 100 growth lines were not dissimilar. However, after 100 growth lines had been deposited, males had a negligible increase in SL while females continued to grow at approximately the same rate with respect to growth line deposition.

## DISCUSSION

The close coupling of visceral and total dry weights strongly suggests that observed differences in TDW (and thus, CSW, CSV and VI) resulted from changes in size-specific visceral weight between months. By comparison, size-specific somatic weight changed little from month-to-month. It is assumed that most of the difference in VDW among samples was due to changes in weight of the gonad, which reflected the reproductive cycle. In the present study, ocean quahogs generally had higher computed VDW in spring and summer than in fall and winter. This result agrees well with those of Jones (1981), Mann (1982) and Rowell et al. (1990), all of whom reported finding greater numbers of quahogs with developing gonads in spring and

TABLE 4.

Analysis of covariance (comparisons of slope and estimated Y) of thirteen different least-squares linear regressions relating X and Y of male and female specimens of *Arctica islandica*. Covariates were linear over the range of values examined in each case. See Table 1 for key to abbreviations of X and Y. NM and NF = number of males and females, respectively, used in regression. ns—cannot reject null hypothesis that there is no difference between males (M) and females (F) in regression statistic ( $p > 0.05$ ); significant differences: \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ . Result indicates the direction of significant differences between the sexes in Y for a given value of X.

X	Y	NM	NF	Slope	Estimated Y	Result
SL	SH	168	218	ns	***	SH M < F
SL	W	168	218	ns	ns	—
SL	ln SW	168	218	ns	**	SW M > F
SL	ln TWW	168	218	ns	ns	—
St	ln TDW	156	200	ns	ns	—
SL	ln SV	168	215	ns	ns	—
SH	ln SW	168	218	ns	***	SW M > F
TWW	SV	168	215	ns	***	SV M < F
TDW	SV	156	198	ns	***	SV M < F
SL	ln SDW	156	200	ns	*	SDW M < F
SL	ln VDW	156	200	ns	***	VDW M > F
SDW	SV	156	198	ns	ns	—
VDW	SV	156	198	*	***	SV M < F

summer, and ripe or spent gonads in fall and winter. In the present study, however, the large variability in size-specific VDW within most of the samples tended to obscure any seasonal cycle. Similarly, the distribution of monthly mean values of CSW, CSV and VI did not reveal a strong seasonal cycle despite the high values in April, June and July.

There are two possible explanations, which are not mutually exclusive, for the lack of significant seasonal differences in condition and calculated tissue weight in the present study: (1) site-specific differences in growth rate (resulting from differences in bottom water temperature, current regime, food supply, etc.) may be obscuring what-

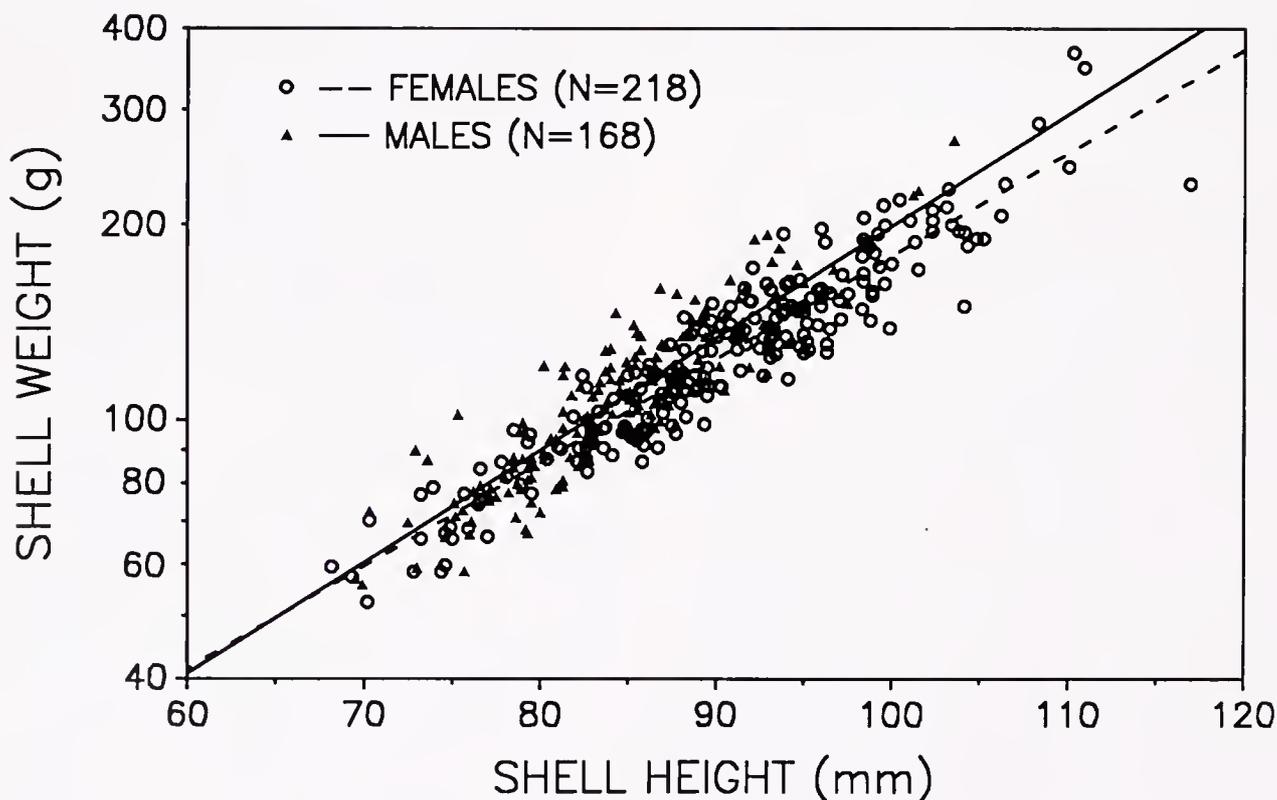


Figure 4. Shell weight plotted against shell height for male and female specimens of *Arctica islandica* collected throughout the course of the study.

TABLE 5.

Results of two canonical discriminant analyses of shell morphometric parameters of male and female *Arctica islandica* (SL = shell length, SH = shell height, W = shell width, SW = shell weight, SV = shell volume). In both cases, the F-statistic suggests significant discriminating power of the derived function, but each yielded only approximately 67% correct sex determinations.

(1) Canonical Variable Y =  $-0.104 (SL) + 0.345 (SH) + 0.064 (W) - 5.612 (\ln SW) + 1.139 (\ln SV) - 2.370$   
 Likelihood Ratio = 0.832; F = 15.159; p = 0.0001  
 Squared Distance Between Male and Female Means = 0.812

Sex	Mean Y	Median Y	Coded As:	Actual Sex:		Total
				M	F	
M	-0.506	-0.611	M	116	74	190
F	0.395	0.394	F	52	141	193
			Total	168	215	383

(2) Canonical Variable Z =  $0.292 (SH) - 5.101 (\ln SW) - 1.375$   
 Likelihood Ratio = 0.843; F = 35.542; p = 0.0001  
 Squared Distance Between Male and Female Means = 0.751

Sex	Mean Z	Median Z	Coded As:	Actual Sex:		Total
				M	F	
M	-0.490	-0.595	M	119	80	199
F	0.377	0.331	F	49	138	187
			Total	168	218	386

ever seasonal trend is evident at a single location; and (2) individual quahogs within a population at a particular site may not be synchronous in their reproductive development each year. Both factors would tend to obscure peaks and troughs in computed indices and tissue weights over the year. In one of the two years in which Jones (1981) sampled populations of quahogs off New Jersey, there were six months (June through October, and December) during which over 40% of the individuals sampled were in a ripe phase of development, while in January, individuals in four different reproductive phases (from ripe to early active) were found. Because his samples were obtained from commercial clammers in New Jersey, site-specific differences as discussed above may be contributing to the lack of synchrony in gametogenic cycles among the populations sampled.

Mann (1982) and Rowell et al. (1990) showed that individual variability in rates of reproductive development may also be high within a population of ocean quahogs. Mann (1982) repeatedly sampled quahogs at specific sites on the Southern New England shelf. Quahogs collected at two sites in June were in all phases of development, from early active through spent. Rowell et al. (1990) also found quahogs in four of five phases of development in April and May at a single site off Nova Scotia, Canada.

In the present study, specimens collected in May may have been slower to develop gonad than those sampled in April, June and July, resulting in lower values of CSV and VI as well as size-specific VDW and TDW. Similarly, quahogs in the August sample may have spawned earlier than those collected in September and October, which also

lowered their computed index and size-specific tissue weight values. Both site-specific and individual differences may have contributed to the lack of seasonal trends in CSV and VI in this study.

Length-specific tissue wet weights in this study were greater than those reported by Ropes (1971) but similar to those reported by Murawski and Serchuk (1979) and Murawski et al. (1982) for quahogs from the mid-Atlantic Bight. Individual quahogs from samples averaging 93 mm in shell length collected in June and July had total wet tissue weights of 36 g in the study of Ropes (1971). In the present study, calculated TWW for 93 mm quahogs in June and July were 48 g, 33% greater than reported by Ropes (1971). Using the shell length-meat weight relationships reported by Murawski and Serchuk (1979) for quahogs collected in January and February off New Jersey, a wet tissue weight of 36 g was calculated for an individual of 95 mm shell length. This compares favorably with the TWW of 38 g and 41 g calculated for quahogs of the same length collected in January and February, respectively, in this study. Similarly, Murawski et al. (1982) reported wet tissue weights of 38 g and 36 g in February and August, respectively, for 95 mm SL quahogs, which agrees well with the 41 g and 39 g TWW calculated for the same months in this study. However, wet tissue weights reported by other researchers were all less than the annual mean TWW observed in this study (44 g for a 95 mm SL quahog).

In the four other studies reporting sex ratios of *Arctica islandica*, two reported parity between the sexes (males:females; 1.09:1, Mann 1982; 0.93:1, Ropes et al. 1984b), while two found significantly more males than females

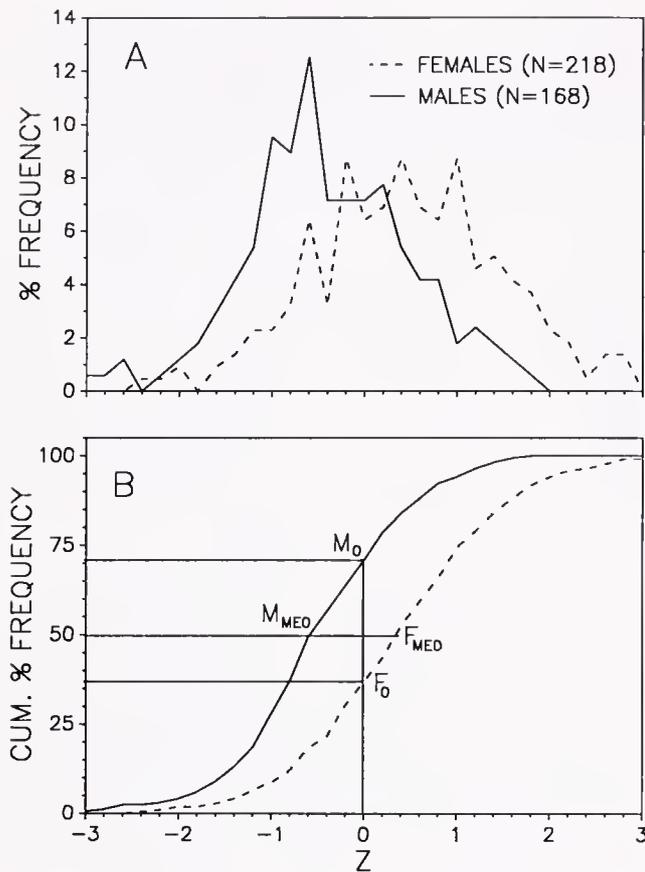


Figure 5. Frequency distribution (A) and cumulative frequency distribution (B) of discriminant variable  $Z$  for male and female specimens of *Arctica islandica*.  $Z$ , a function of shell weight and height, should be negative for males and positive for females; it is described further in text.  $M_{MED}$  and  $F_{MED}$  are the medians of the male and female  $Z$  distributions, respectively (Table 5). The locations of  $M_0$  and  $F_0$  indicate that 70.8% of the males had negative  $Z$  values, and 63.3% of the females had positive  $Z$  values.

(1.35:1, Jones 1981; 2:1, Rowell et al. 1990). In the present study, significantly fewer males than females were found (0.77:1). With respect to similarities in size distribution and locations of catch, samples in this study were most similar to those of Jones (1981), yet each yielded quite different sex ratios. If only data for quahogs 70 mm shell length and greater are compared, results in this study are similar to those of Ropes et al. (1984b; 0.67:1), but very different from those of Rowell et al. (1990; 2.31:1). Reasons for these differences in reported sex ratios even between samples with similar size ranges are not known. Evidence for environmental determination of sex, hermaphroditism, ambisexuality and protandry are all lacking for this species.

Despite the significant differences between males and females in the relationship between shell length and height, the two variables alone were poor discriminators of sex. Use of either canonical variable  $Y$  (based on all five shell morphometric parameters) or  $Z$  (based on shell height and weight) to determine sex was only 17% more accurate than pure chance. Considering the ease with which sex can be determined by gonad biopsy, the utility of canonical discriminant variables  $Y$  and  $Z$  may be limited, especially considering that the animal must be sacrificed to compute shell weight. Attempts to discriminate sex based only on shell length, height and width (which would not require sacrificing the animal) were unsuccessful.

To explain the greater size of female than male specimens of *Arctica islandica* in his study, Ropes et al. (1984b) suggested that females have greater longevity than males. This hypothesis is supported by the data on size and number of growth lines in the present study, if it is assumed that growth lines are formed at the same rate by both sexes, as Ropes et al. (1984b) did. Several researchers have concluded that growth line formation, which occurs in fall and early winter in *A. islandica*, is linked to the reproductive

TABLE 6.

Best-fit equations relating shell length (SL) and number of growth lines (GL) of female ( $N = 22$ ) and male ( $N = 25$ ) ocean quahogs. Both equations were generated using SYSTAT (Wilkinson 1988). For females, a linear model (ANOVA; SYSTAT module MGLH) yielded the best fit; for males, a non-linear model of the form,  $SL = B + Ce^{K(GL)}$  (SYSTAT module NONLIN), yielded the best fit; SE = standard error; other abbreviations as in Tables 2 and 3.

A. Females:  $SL = 82.43 + 0.17 (GL)$ ;  $r = 0.88$

Source of Variation	SS	df	MS	F	p
Regression	1514.23	1	1514.23	69.63	$p < 0.001$
Residual	434.95	20	21.75		

B. Males:  $SL = 96.92 - 60.60 e^{-0.04(GL)}$ ; final loss = 291.55

Parameter	Estimate	SE
B	96.92	1.16
C	-60.60	23.28
K	-0.04	0.01

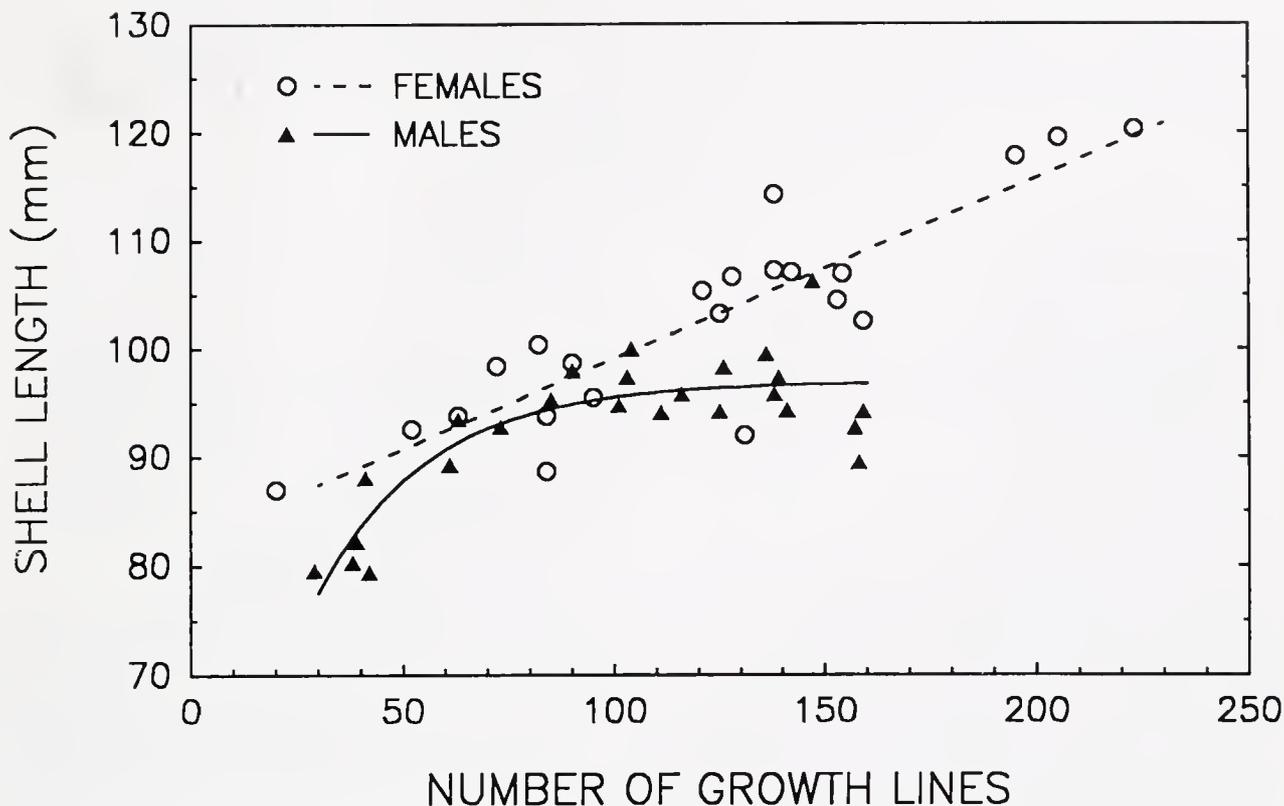


Figure 6. Shell length plotted against number of growth lines in radial shell sections of male ( $N = 25$ ) and female ( $N = 22$ ) specimens of *Arctica islandica*. Dashed and solid lines describe growth of males and females according to the equations in Table 6.

cycle (Thompson et al. 1980), and specifically to spawning (Jones 1980; Ropes et al. 1984a, b). Mann (1982) suggested that multiple spawnings in a single year were possible, but that gonad maturation probably occurred only once each year in individuals of both sexes. Thus, if growth line formation is linked to spawning, then more than one growth line could be formed in some years. Results of recent *in situ* growth studies (Lutz et al. 1989) support this claim, albeit with only a limited number of specimens. If specimens can form more than one growth line per year, this could reduce the mean age of the exploited population in the mid-Atlantic Bight, and thus, have profound implications on management of this important commercial species.

It may not be necessary to hypothesize a difference between the sexes in periodicity of growth line formation to explain the differences between the female and male "growth" curves in Figure 6. If females formed significantly more than one growth line per year, then the older, larger specimens would be significantly younger than the number of growth lines counted, moving the specimens plotted on the right portion of the graph to the left on a plot of SL against age. This would have the effect of either increasing the slope of the line if the relationship were still linear, or possibly yielding an exponentially increasing

function. The former would yield even greater differences between the sexes in size at age than if an annual periodicity of growth line formation is assumed, while the latter possibility can be rejected on bioenergetic considerations. If males formed significantly more than one growth line per year, then the new function relating SL and age could be linear, with a slope similar to that relating SL and number of growth lines for females. Thus, growth rates of the two sexes could be similar, but females would still have greater longevity and a larger asymptotic size regardless of the periodicity of growth line formation. The fact that males had a higher VI throughout the year as well as greater size-specific VDW than females in this study suggests that males expend a greater percentage of their total available energy on gonad production than females. This difference in energy expenditure may be partly responsible for the greater longevity and size of female than male ocean quahogs observed in this study and others (Ropes et al. 1984b; Rowell et al. 1990).

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## DISEASES AND PARASITES OF MUSSELS (*MYTILUS EDULIS*, LINNEAUS, 1758) FROM TWO SITES ON THE EAST COAST OF THE UNITED STATES

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**ABSTRACT** Samples of blue mussels were collected from New Jersey and Maine, on the east coast of the United States, and examined for histopathology and parasites. Samples were collected every 3 months and several previously unreported parasites were observed: a microcell-like (Apicomplexa) parasite and a haplosporidian-like (Asctospora); and an intracellular ciliate of the digestive tubules. The only parasite which elicited a marked host hemocyte response was the microcell-like parasite, which occurred in a single animal collected from New Jersey.

**KEY WORDS:** mussel, histopathology, parasite, disease

### INTRODUCTION

Although mussel populations are not documented as having experienced the number and severity of mass mortalities recorded for various oyster species, there are several records citing major die-offs of cultured and wild populations (Korringa 1951, Li et al. 1979, Munford et al. 1981). In most of these cases, knowledge of the disease and parasite status prior to the disease outbreak is negligible. This lack of information can make interpretation of the cause of the disease outbreak and subsequent control, or reversal of the spread of the disease, very difficult. Since aquaculture of blue mussels in the northeastern waters of the US and Canada has developed rapidly over the last 10 years (Lutz et al. 1989) and is now a multi-million dollar industry, there is a paralleled increase in concern over the lack of base-line information about which parasites are already present, where they are distributed and their pathological significance. In 1985 and 1986, therefore, we initiated such a survey and the summary of our findings are presented in this research note. This is an intensive histological evaluation of a small number of mussels collected from two survey sites and is not intended to be an exhaustive survey. It is hoped, however, that these initial findings will be of use to future larger-scale sampling programs.

### MATERIALS AND METHODS

Between June 1985 and February 1986 a total of 201 blue mussel, *Mytilus edulis*, were collected from subtidal populations at Boothbay Harbor, Maine (106), and Manasquan Sea Port, New Jersey (93). Samples of 21-33 animals from Maine and samples of 17-26 animals from New Jersey were collected every three months (Table 1). The sizes of the samples ranged from 3.5 to 6.5 cm in total length. All animals were processed within 24 hours of removal from the water. Transverse tissue sections, including

digestive gland, mantle, gills, gonad and foot were fixed in Davidson's fixative (Shaw and Battle 1957) for 24 hours. The fixed tissues were infiltrated with paraffin wax and 5  $\mu$ m sections stained with iron hematoxylin, acid fuchsin and aniline blue (Gray 1954). Microscopic analysis was carried out at low and high dry magnification, as well as under oil immersion and approximately one half hour was devoted to each slide. Observations were analysed using a two way ANOVA for comparison of the frequency of appearance of each parasite with sample site and date of collection.

### RESULTS

A summary of the histological observations collected from the mussel samples is presented in Tables 2A and 2B.

Hemocyte infiltration of the connective tissue appeared to increase in frequency in both Maine and New Jersey locations, with consistently higher frequencies in Maine.

The infiltrating hemocytes had large nuclei and a prominent nucleolus. In the spring sample from Maine a mussel was found with abnormally large hemocytes. These hemocytes had a dense cytoplasm, large nucleus and nucleoli (Fig. 1) and frequently included mitotic figures. These hemocytes are identical to those observed in blue mussels from the Baltic Sea (Figueras unpublished data). These hemocytes also resemble those described as being neoplastic in blue mussels from the United Kingdom (Green and Alderman 1983) and the western coast of the United States (Elston et al. 1988).

Rickettsiae-like inclusions were found in the epithelial cells of the digestive tubules in all samples collected. These appear to cause a small increase in the size of the cell, but there does not appear to be a marked host cell response to the infection.

Another common parasite of the digestive tubule epithelia was a ciliate protozoan (Fig. 2). These ciliates were



TABLE 1.

Date, location and sample-sizes used for this investigation.

Place	Date			
	1985		1986	
	June 7	August 28	Nov. 18	Feb. 28
Boothbay Harbor Maine	21	26	26	33
Manasquan Sea Port New Jersey	17	26	26	26

consistently found within the tubule epithelial cells, which is a highly unusual infection site for bivalve ciliates which are most commonly found extracellularly or on the surface of the gills and mantle (Fenchel 1965). Similar parasites have been observed in blue mussels from eastern Canada (McGladdery,<sup>1</sup> personal communication). Despite their intracellular location, these ciliates do not appear to provoke a specific host response. The frequency of these ciliates in the histological sections was greatest in the June samples (Table 2A and 2B), with no significant difference between sample sites.

*Steinhausia mytilovum* was found in two samples at each

TABLE 2A.

Prevalences (%) of the different parasites and lesions from Maine.

Organ	Parasite or Lesion	Maine Samples			
		1	2	3	4
Mantle	Infiltration	4	35	33	—
	Steinhausia	—	20	4	—
	Enlarged hemocytes	—	—	—	3
	Ciliates	—	—	—	—
Gills	Hermaphrod	—	—	—	3
	Ciliates	76	85	50	36
	Bacterial cyst	5	—	—	—
	Haplospor.	4	—	—	—
Kidney	Trematode	—	—	—	—
	Ciliates	10	—	4	9
	Haplospor.	—	4	—	—
	Infiltration	—	—	4	—
Dig gland	Trematode	—	—	—	—
	Rickettsiae	14	31	31	9
	Ciliates	—	4	—	—
	Intracell. ciliate	—	20	—	9
	Crustacean	—	4	8	—
	Infiltration	—	—	—	12
	Neoplasias	—	—	—	3
	Trematode	—	—	—	—
Foot	Haplospor.	—	12	—	—
	Cyst	50	23	8	—
	Infiltration	—	—	19	3

place. The maximum value of the prevalence was 20% in Maine. In the New Jersey samples only 4% of the females were infected in each sample.

*Proctoeces maculatus* was the only trematode found, and occurred in the August sample collected from New Jersey. It appears to be located predominantly within the digestive gland, however, in a heavier infection it was observed in gill tissue (Fig. 3), kidney, mantle and between the follicles of the gonad. The degree of hemocytic response to the sporocyst infection was highly variable in the samples examined. *P. maculatus* was the only parasite which showed a significant difference ( $F = 9.77$ ,  $df = 1$ ) between frequencies at each sampling location.

A haplosporidian-like protozoan was found in the sample collected in August at the Maine location. The plasmodia were detected in the connective tissue between the digestive tubules (Fig. 4a), on the tips of the gills and in the external epithelium of the mantle (Fig. 4b). The plasmodia measured from 18  $\mu\text{m}$  of mean diameter ( $n = 20$  S.D.  $\pm 1$ ) and contained from 5 to 60 nuclei. There was no detectable host response to this parasite.

A microcell-like organism which closely resembles *Bonamia* in histological section was found in a single mussel from New Jersey, collected in February. What appear to be single-cell as well as plasmodial stages were

TABLE 2B.

Prevalences (%) of the different parasites and lesions from New Jersey.

Organ	Parasite or Lesion	New Jersey Samples			
		1	2	3	4
Mantle	Infiltration	—	—	19	8
	Steinhausia	—	4	4	—
	Enlarged hemocytes	—	—	—	—
	Ciliates	—	—	—	—
Gills	Hermaphrod	—	—	—	—
	Ciliates	82	27	23	21
	Bacterial cyst	—	—	—	—
	Haplospor.	—	—	—	—
Kidney	Trematode	—	8	—	—
	Ciliates	18	—	—	4
	Haplospor.	—	—	—	—
	Infiltration	—	—	4	4
Dig gland	Trematode	—	11	—	8
	Rickettsiae	12	46	4	4
	Ciliates	—	—	—	4
	Intracell. ciliate	—	—	8	—
Crustacean	Crustacean	—	—	—	—
	Infiltration	—	—	27	8
	Neoplasias	—	—	—	—
	Trematode	12	50	19	23
Foot	Haplospor.	—	—	—	—
	Cyst	9	52	19	15
	Infiltration	—	—	4	4





Figure 1. "Neoplastic" blood cells.

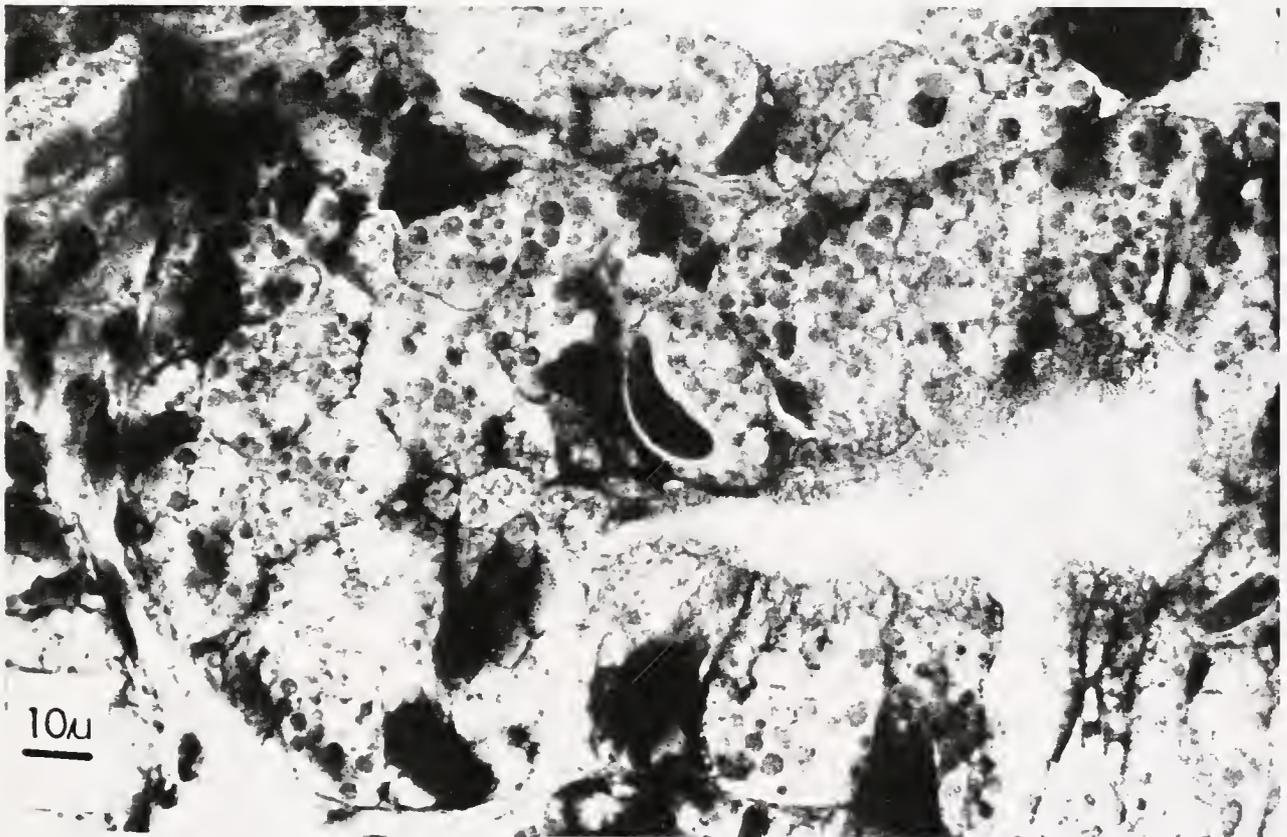


Figure 2. Small protozoan parasite of the cells of the digestive tubules.



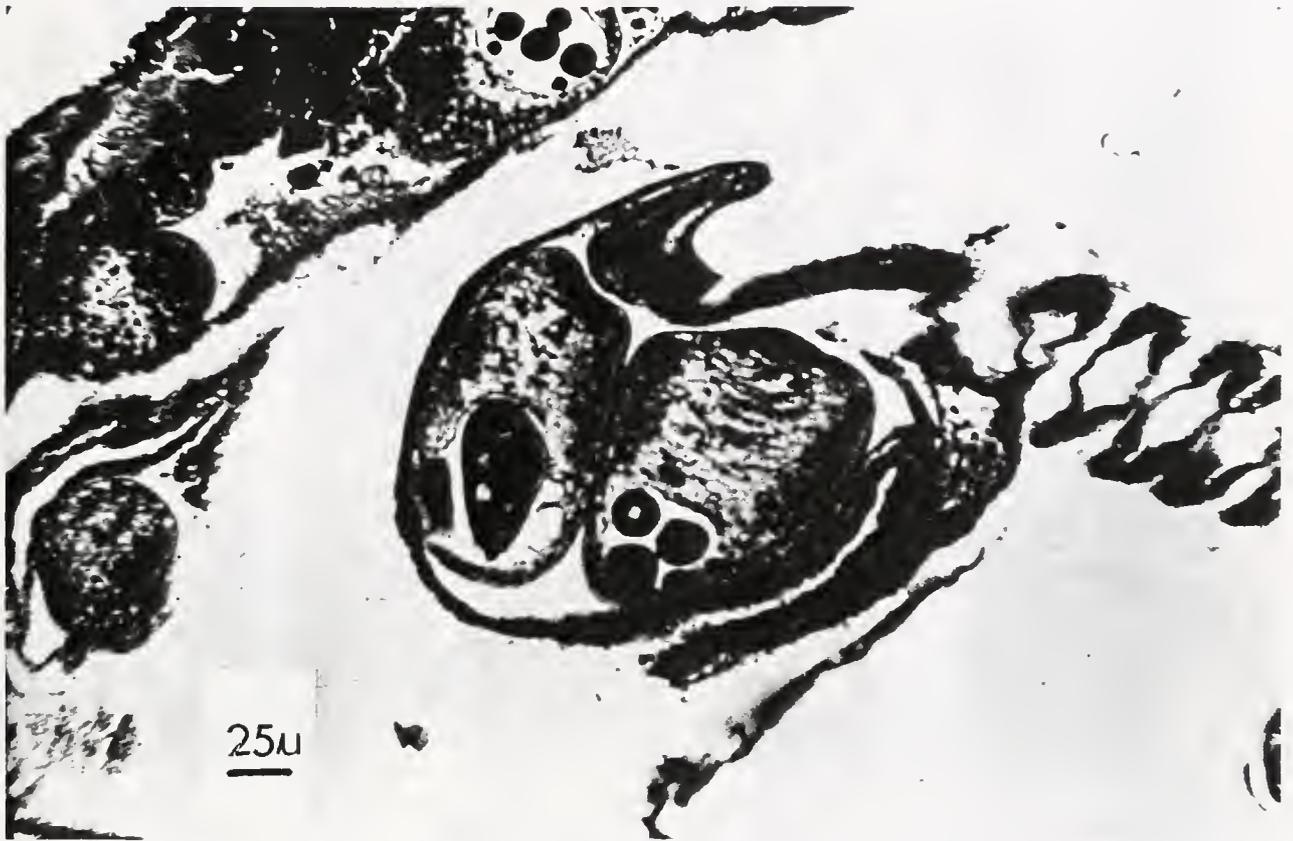


Figure 3. Trematodes were found in several organs such as the gills.

found (Fig. 5). A very intense hemocyte response appeared to be elicited by this infection. The average dimensions of these microcell organisms were ( $n = 20$ )  $2 \mu\text{m}$  for the single cells and  $10 \mu\text{m}$  for the plasmodial stage. The ultrastructure and monoclonal antibody characteristics of this "mussel microcell" are currently being investigated.

The most consistent parasite found in the mussel survey was the gill ciliate *Ancistrum mytili*, which was present in all the samples examined. The frequencies were greatest in August at the Maine site, while mussels from New Jersey showed the highest frequency in June. Both locations showed a decline in gill-ciliate frequency in the November and February samples, which may indicate a seasonal infestation pattern. There was no marked host-response to these gill ciliates, even in the more heavily infested summer samples. It does not appear, therefore, that these ciliates were causing any irritation of the gill surface.

#### DISCUSSION

This type of base-line study is a preliminary overview of what is present in small samples taken from two mussel populations where no mass mortalities were detected. Bearing in mind the small sample size, it is interesting to note that several previously unreported parasites in these areas, were observed, at least two of which may be related to known shellfish pathogens (namely the microcell-like

protozoan and the haplosporidian-like protozoan). Farley (pers. comm) found a *Minchinia* in mussels from Damascotta River (Maine). These observations are worth noting for several reasons: firstly, the detection of these parasites both in small samples and using histology as the monitoring tool, means that they can be present in greater numbers than reported here. The fact that they have not been previously reported also indicates that routine histological observations of several minutes a slide may not be sufficient to detect the smaller parasites, such as microcells. Such observations, although appreciably tedious, are required in order to get an accurate picture of what is present in a healthy population. Parasites are far easier to detect in diseased animals when, more often than not, the disease-organism is beyond control. A detailed survey of healthy populations, starting small, and covering the annual development cycle of the shellfish, is highly recommended to provide accurate information which may be invaluable in the analysis of any future disease problem.

#### ACKNOWLEDGMENTS

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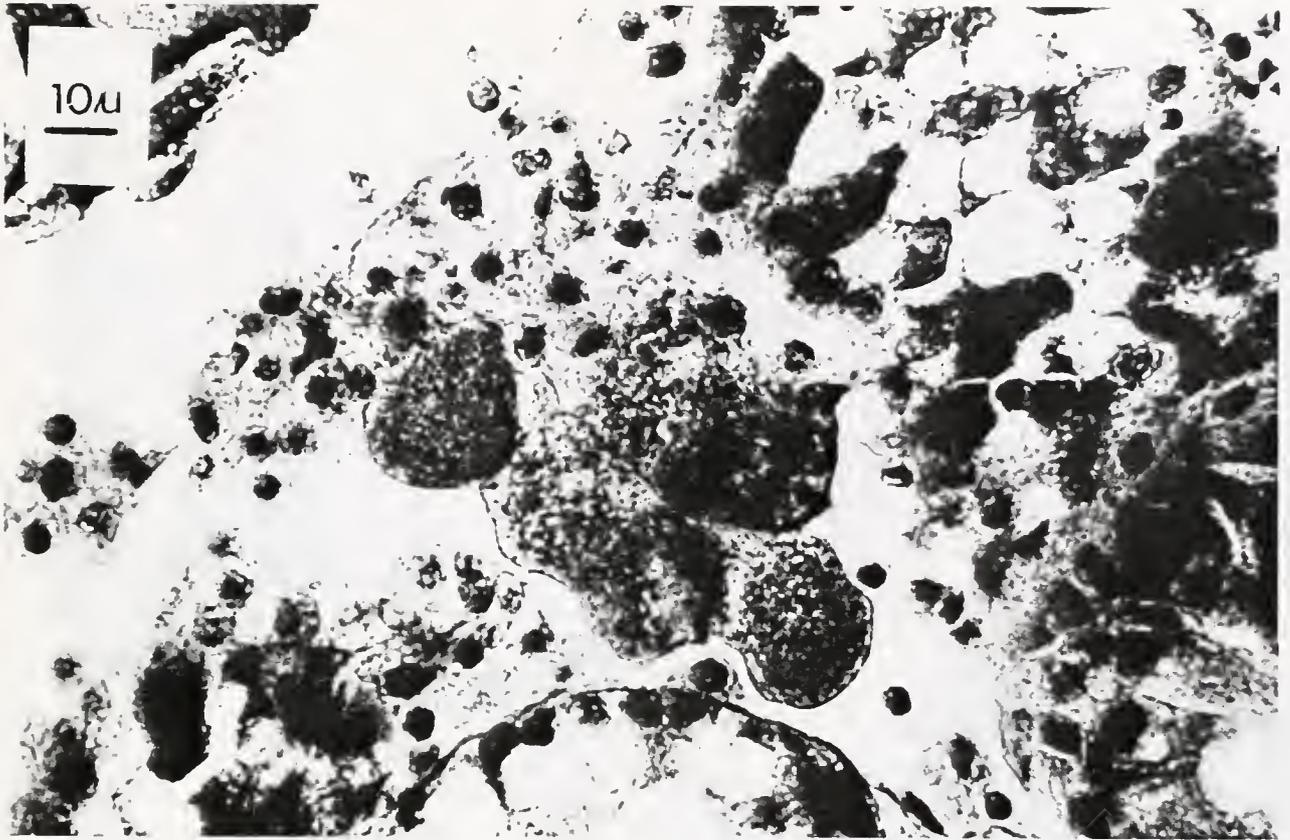


Figure 4A, 4B. A haplosporidian was found in the samples from Maine with a low prevalence.



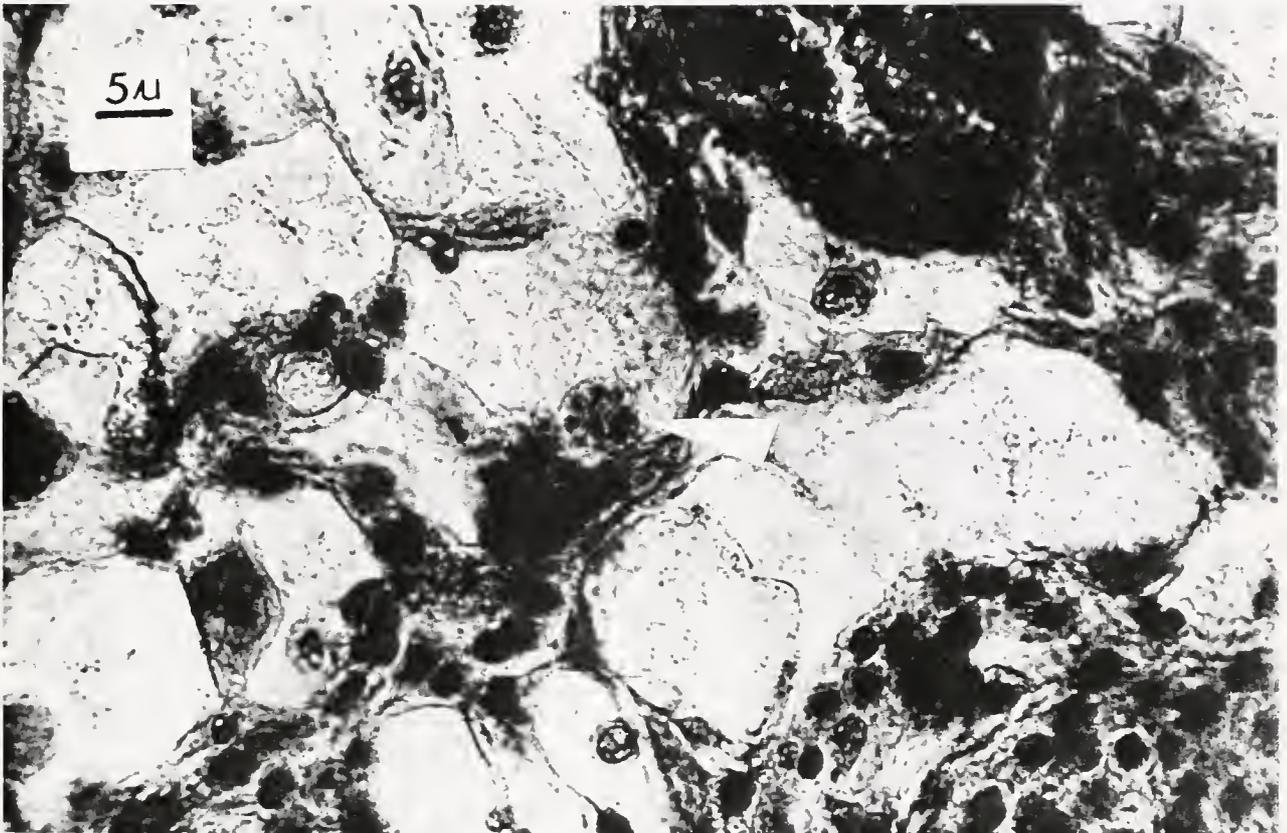


Figure 5. An organism similar to *Bonamia* was found in a mussel from New Jersey.

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## EFFECTS OF IMMERSION TIME AND TIDAL POSITION ON *IN SITU* GROWTH RATES OF NATURALLY SETTLED EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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**ABSTRACT** In order for an intertidal suspension-feeding bivalve species to maintain equal (or greater) rates of growth as their subtidal siblings, a) some degree of adaptive metabolic capacity must exist in the intertidal populations to compensate for shorter feeding periods and/or b) some selective disadvantage, such as predation or disease, must exist in the subtidal populations. The purpose of this study was to determine the effects of immersion time on juvenile *in situ* growth rates of *Crassostrea virginica* which underwent natural metamorphosis in tidal creeks of a South Carolina salt marsh. Results indicate differential rates of growth within and between intertidal and subtidal oysters over time. The critical point of differential growth rates between subtidal and intertidal spat began immediately post-metamorphosis and is likely due, initially, to the greater immersion time and, thus, greater time for energy acquisition by the subtidal spat. This relationship did not remain constant throughout our study, however. Subtidal spat appear to have an early advantage of greater energy acquisition which is manifested in the achievement of greater size in less days, although a significant controlling factor for growth during this early postmetamorphosis stage was immersion time. A shift in relative growth rates between intertidal and subtidal spat occurs at ~850 h of post-settlement immersion, when intertidal rates surpass those of the subtidal population. Intertidal spat were able to compensate for an initially lower growth rate by steadily increasing it over time. A dramatic shift from a rapid rate of growth in the early/mid juvenile stage to a much slower rate of growth requires less days to occur for subtidal than intertidal spat, with subtidal populations exhibiting a plateau in growth at ~1320 h of post-settlement immersion. No significant difference in size between populations remained at this point. We hypothesize that this shift in growth rate occurs at some genetically pre-determined size and is due to fluctuations in anabolic processes resulting in temporary reallocation of production from principally shell growth to primarily soft tissue growth. The significant increase in intertidal growth rate with time may be due to natural selection for survival of individuals capable of greater assimilation efficiency than generally found in subtidal spat. Calculations demonstrate that differences in feeding time alone were insufficient to explain completely the reduced intertidal growth. Analysis of mean maximum age of spat indicated significantly greater mortality in intertidal versus subtidal spat. Our results led us to hypothesize that intertidal spat surviving to ~7 wk post-settlement possessed some genetically pre-determined compensatory capability for reduced feeding time.

**KEY WORDS:** bivalve, compensation, *Crassostrea virginica*, growth rate, immersion time, intertidal, spat, subtidal

### INTRODUCTION

In South Carolina, approximately 95% of the natural oyster (*Crassostrea virginica*) grounds are intertidal (Dame 1979). The success of intertidal oysters in the South Atlantic United States and the lack of subtidal oysters may be due to predators, boring sponges, and annelid worms which can cause considerable damage to subtidal oysters (Burrell 1986). Intertidal zones may also offer more suitable substrates, more abundant food, higher recruitment and less turbidity along with greater current velocities and less siltation. Oyster growth is continuous throughout the year in the southeast U.S. and is most rapid in the summer (Ingle 1950), although it slows during midwinter in South Carolina (Burrell et al. 1981). The ability to determine the optimal combination of environmental parameters that will yield the highest rate of growth is of paramount importance to the industrial culturing of bivalves. A great deal is already known about bivalve dietary requirements (i.e., Pruder et al. 1982). A number of studies have also been

conducted with respect to bivalve growth rates as a function of subtidal culturing (Shaw 1966, Shaw and Merrill 1966, Manzi et al. 1977, Singarajah 1980, MacDonald and Bourne 1989, Grant et al. 1990), distance from shore (Ambrose et al. 1980, Dayton et al. 1989), salinity (Shaw 1966), subtidal water column depth (Duggan 1973), and genetic make-up (Losee 1979; Paynter and Dimichele 1990). There also are a number of studies dealing with intertidal vs. subtidal bivalve growth rates (Eldridge et al. 1979, Breed-Willeke and Hancock 1980).

If an intertidal suspension-feeding bivalve species is able to maintain equal (or greater) rates of growth as their subtidal siblings, a) some degree of adaptive metabolic capacity must exist in the intertidal populations to compensate for shorter feeding periods and/or b) some selective disadvantage, such as predation, disease or lower food quality/quantity, must exist in the subtidal populations. Possible adaptive metabolic activities may be broadly grouped as energy-conserving or energy-supplementing (Gillmor 1982). Although numerous combinations of specific metabolic adaptations and selective disadvantages are possible, one need only study rates of growth in inter-

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versus subtidal suspension-feeding sibling bivalve populations under the same environmental conditions (other than immersion time) to demonstrate whether such adaptive metabolic capacities or selective disadvantages exists for a given species. Growth has been reported to be greater in subtidal *Crassostrea virginica* than for intertidal beds in South Carolina (Burrell 1982). Growth rates for *Crassostrea gigas* (initial 2–4 cm height) have been reported to be greater in subtidal than intertidal culture, despite excessive fouling of subtidal trays (Michael and Chew 1976). However, Gillmor (1982) found that *C. virginica* growth was better per unit immersion time at depths below mean high water in the intertidal than in subtidal beds. The equivocal nature of conclusions from studies such as these, concerning growth rates of intertidal vs. subtidal oysters, led to the implementation of our research. The purpose of our study was to determine the effects of immersion time on juvenile *in situ* growth rates of *C. virginica* which metamorphosed naturally in tidal creeks of a South Carolina salt marsh.

#### MATERIALS AND METHODS

Asbestos spat collecting plates (~15<sup>2</sup> cm) were suspended horizontally at a site in a tidal creek of the North Inlet salt marsh estuary, near Georgetown, South Carolina, in 1987 and 1989. The suspension apparatus consisted of four replicate rope harnesses hung from poles driven into the bottom of a creek ~2 m from the creek bank where abundant oyster reefs exist. Each harness line was anchored with a large cement block and held a collecting plate at both 30 cm above and below mean low tide. The average annual tidal range in North Inlet is ~1.4 m. At the beginning of each year's study (early June), clean plates were allowed to remain in the field harnesses at their respective tidal elevations for 7 and 14 days (in 1987 and 1989, respectively) in order for newly metamorphosed ("spat") *Crassostrea virginica* to attach. At the end of the initial settling periods, each plate was removed and observed with a dissecting microscope. The location of several spat, widely spaced from each other across the bottom surface of each plate, was marked on a transparent overlay. Any epibionts near one of these spat were removed. The heights (maximum dorsal to ventral distance) of individual spat were measured via an ocular micrometer and the plate replaced to its original tidal position. All measurements of spat occurred at the field site so that the plates were out of their harnesses for only ~1 to 1.5 h. Once the spat had grown to ~20 mm, measurements were made macroscopically with a small flexible ruler. Measurements were made from June through September at 6 to 9 day intervals, with the exception of the final interval of 1989 which was 13 days. Daily immersion times for the intertidal plates were calculated using a computer simulated tidal model derived from data gathered as part of the ongoing NSF sponsored long-term ecological research that has been conducted in North Inlet for the last 10 years (Sklar and Childers, unpubl.

data). Intertidal spat were exposed ~20% of the tidal cycle. Average monthly water temperatures range from ~26 to 28°C during June to September, while average monthly air temperatures range from ~22 to 32°C.

All statistical analyses were performed with a Macintosh SE computer using the StatView 512+ statistical package (Gagnon and Feldman 1986), with level of significance set at  $p \leq 0.05$ . Analyses of variance (ANOVAs) were performed to ascertain differences between subtidal and intertidal instantaneous cumulative rate of growth (RATE<sub>c</sub>, eq-1), instantaneous periodic rate of growth (RATE<sub>p</sub>, eq-2), and square root-transformed height.

$$\text{RATE}_c = \text{HT}_{(t=z)} - \text{HT}_{(t=0)} / \text{Total days measured} \quad (1)$$

$$\text{RATE}_p = \text{HT}_{(t=z)} - \text{HT}_{(t=x)} / \text{Period days measured} \quad (2)$$

where

HT<sub>(t=z)</sub> = spat height (mm) at the end of a given time period,

HT<sub>(t=0)</sub> = initial spat height (mm) at beginning of study, and

HT<sub>(t=x)</sub> = initial height (mm) at beginning of a given time period.

Kendall's coefficient of rank analyses were employed to detect any significant ( $p \leq 0.05$ ) correlations of spat shell height, cumulative growth rate (Equation 1) and periodic growth rate (Equation 2) with total cumulative hours of post-settlement immersion (HRS<sub>t</sub>) and the hours of immersion for a given growth period (HRS<sub>p</sub>), usually 6 to 9 days. Linear and polynomial regression analyses were then carried out to further elucidate the nature of any significant relationships. Mann-Whitney U analysis was utilized to determine differences in survival between subtidal and intertidal spat. Due to the manner in which spat were allowed to initially settle on the collecting plates (see above), the maximum age of spat at the time of initial measurement ranged from a minimum of 1 to a maximum of 13 days. Thus, maximum estimated age of any given spat at the time of each measurement was the sum of its maximum initial age plus the number of days subsequent to its initial measurement. Since it was not possible to calculate mortality from initial setting of spat to our initial measurement time, all mortality rates were calculated only for spat which initially survived 2 wks (based on maximum estimated age) on the collecting plates. Mortality rates were calculated for subtidal and intertidal spat as given in Equation 3. Total initial numbers of subtidal and intertidal spat for mortality calculations was 33 and 19, respectively.

$$\text{Cumulative \% mortality} = [(\text{spat}_{t=0} - (\text{spat}_{t=1,2,3 \dots z})) / \text{spat}_{t=0}] 100 \quad (3)$$

where

(spat<sub>t=0</sub>) = # of spat at the initial measurement period, and

(spat<sub>t=1,2,3 \dots z</sub>) = # of spat at the end of a given time period.

## RESULTS

Significant positive correlations (via Kendall's coefficient of rank) were found to exist for both spat height ( $\tau = 0.74$ ,  $p < 0.001$ ) and cumulative growth rate ( $\tau = 0.24$ ,  $p < 0.001$ ) with  $HRS_t$ . The spat periodic growth rate was significantly correlated with  $HRS_p$  ( $\tau = 0.20$ ,  $p < 0.001$ ). Analysis of variance (ANOVA) detected no significant difference for square root of spat height on the initial day of measurements, nor for mean spat periodic growth rate, between intertidal and subtidal plates. However, ANOVA did yield a significant ( $p = 0.0001$ ) difference between the mean square root of spat height of all intertidal (mean = 2.72 mm, SE = 0.18) versus subtidal (mean = 3.84 mm, SE = 0.13) measurements. A significant ( $p = 0.0001$ ) difference was also found (via ANOVA) between the mean cumulative growth rate of intertidal (mean = 0.27 mm day<sup>-1</sup>, SE = 0.02) versus subtidal (mean = 0.51, SE = 0.02) spat. When cumulative growth rates of intertidal and subtidal spat were separately regressed on  $HRS_t$  no significant relationship was found for subtidal spat. Linear regression of intertidal cumulative growth rate, however, was significantly ( $r = 0.65$ ,  $p = 0.0001$ ) correlated with  $HRS_t$ . Further analysis via third order polynomial regression (Fig. 1) demonstrated a plateau in the increase of intertidal cumulative growth rate with increased  $HRS_t$ . Additional ANOVA also demonstrated no significant differences between subtidal and intertidal cumulative growth rates at  $\geq 850$   $HRS_t$ . When periodic growth rate was examined at  $\leq 850$   $HRS_t$ , subtidal spat (mean = 0.47 mm day<sup>-1</sup>, SE =

0.04) were significantly greater ( $p = 0.0003$ ) than intertidal spat (mean = 0.28 mm day<sup>-1</sup>, SE = 0.04). At  $\geq 850$   $HRS_t$ , intertidal spat (mean 0.63 mm day<sup>-1</sup>, SE = 0.10) had a significantly greater ( $p = 0.0079$ ) periodic growth rate than subtidal (mean = 0.36 mm day<sup>-1</sup>, SE = 0.04).

Separate regression analyses of square root of intertidal and subtidal spat heights with days measured both yielded significant ( $p = 0.0001$ ) relationships (Fig. 2a and b). Analysis by the GT2-method for testing slopes (Sokal and Rohlf 1981) demonstrated no significant difference between the slopes of these two regression lines. However, T-test analysis of intercepts (Kleinbaum and Kupper 1978) indicated a significantly ( $p < 0.001$ ) greater intercept for the subtidal (1.886 mm) than the intertidal (1.187 mm) model. Graphical illustration (Fig. 2b) indicated that the relationship between square root of height and days measured may be better explained by curvilinear rather than linear regression for subtidal spat. The resulting second order polynomial regression (Fig. 3) explains a greater amount of the size variability (86%) compared to the previous linear model (79%). Regression line slope of square root of subtidal spat height with days measured was significantly greater ( $p < 0.001$ ;  $\text{sqrtHT} = 0.107$  [days] + 1.463) than the intertidal model ( $\text{sqrtHT} = 0.026$  [days] + 4.249) when data was restricted to  $\leq 850$   $HRS_t$ , but significantly less ( $p < 0.001$ ;  $\text{sqrtHT} = 0.069$  [days] + 1.161) than the intertidal model ( $\text{sqrtHT} = 0.064$  [days] + 1.264) when data was restricted to  $\geq 850$   $HRS_t$ . When restricted to  $\leq 850$   $HRS_t$ , the intercepts of the subtidal and intertidal regression models were not significantly different (T-test for

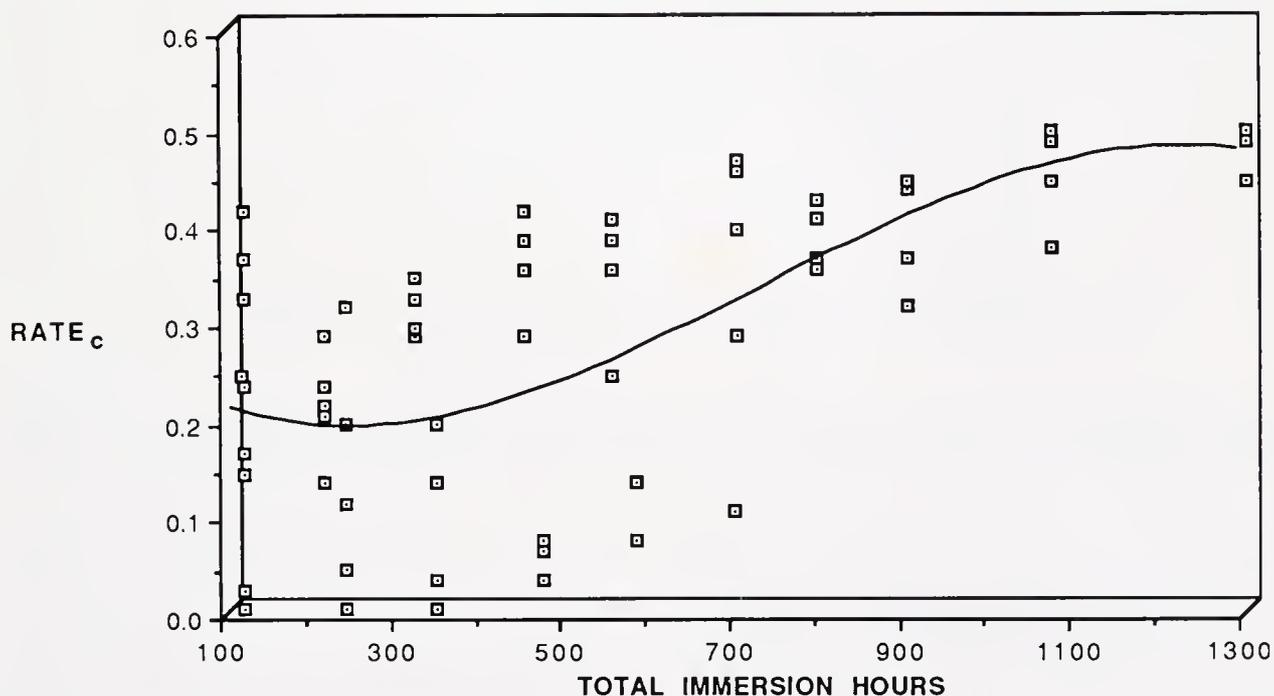


Figure 1. Polynomial regression of cumulative instantaneous growth rate ( $RATE_c$ ) for intertidal spat as a function of total immersion time ( $HRS_t$  in h). Regression equation is as follows:  $RATE_c = 0.25 - (5.33 \times 10^{-4})(HRS_t) + (1.33 \times 10^{-6})(HRS_t)^2 - (6.09 \times 10^{-10})(HRS_t)^3$ ;  $p = 0.0001$ ;  $r = 0.653$ .

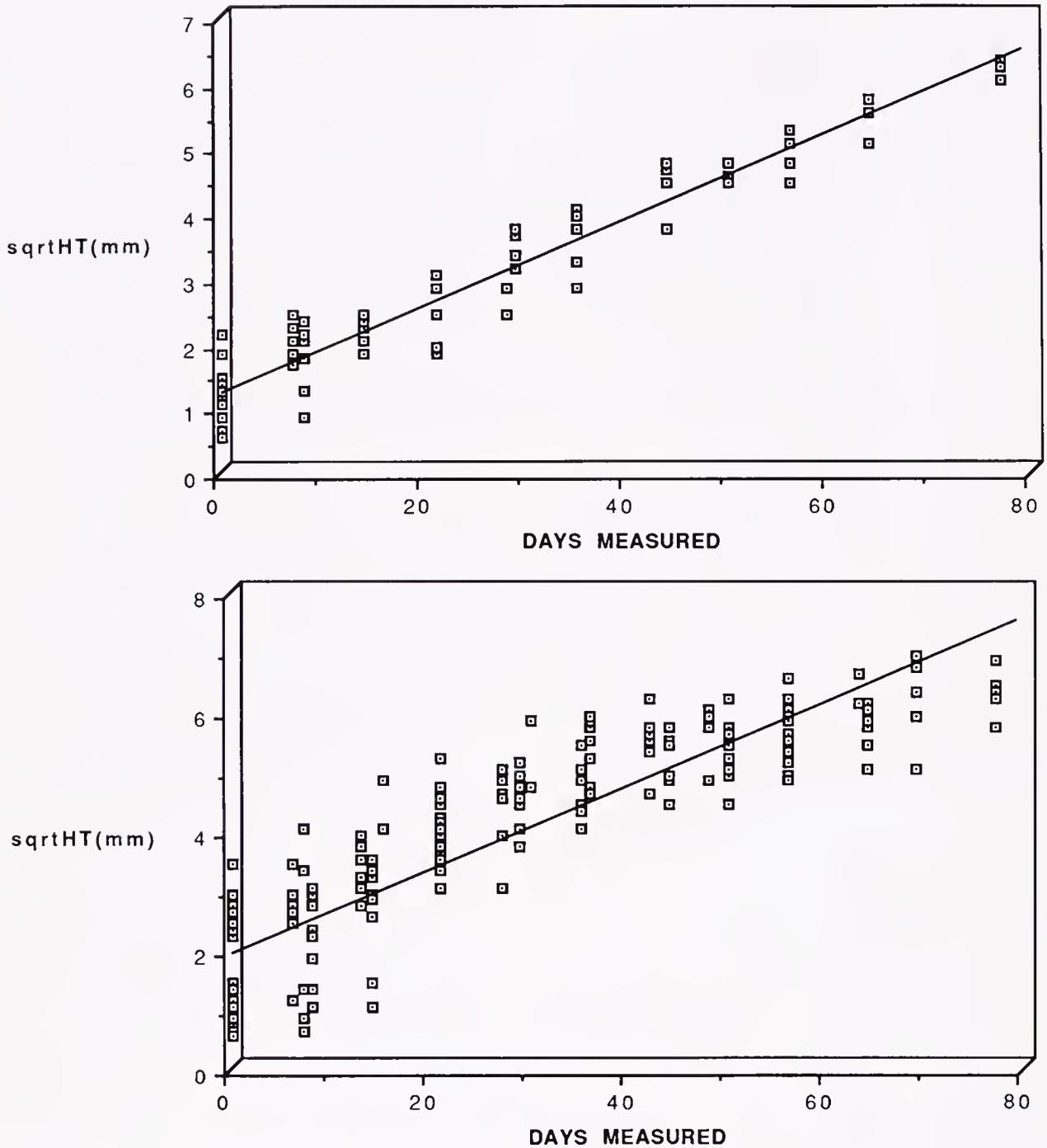


Figure 2. a) Linear regression of square root of intertidal spat height (sqrtHT) as a function of days measured. Regression equation is as follows:  $\text{sqrtHT}(\text{mm}) = 1.18 + (6.68 \times 10^{-2})(\text{days})$ ;  $p = 0.0001$ ;  $r = 0.968$ . b) Linear regression of square root of subtidal spat height (sqrtHT) as a function of days measured. Regression equation is as follows:  $\text{sqrtHT}(\text{mm}) = 1.87 + (7.06 \times 10^{-2})(\text{days})$ ;  $p = 0.0001$ ;  $r = 0.890$ .

intercepts). When restricted to  $\geq 850 \text{ HRS}_t$ , the intercept was significantly greater ( $p < 0.05$ ) for the subtidal model.

Mann-Whitney U analyses indicated that a significantly ( $z$ , corrected for ties = 2.902,  $0.01 > p > 0.001$ ) greater mean maximum estimated age was attained by subtidal (mean = 45.4 days, SE = 1.8) versus intertidal (mean = 36.6 days, SE = 2.6) spat. Mortality of intertidal spat pla-

teaued at 79% and maximum estimated age of 7 wks, while subtidal mortality plateaued at 54.5% and 8 wks maximum estimated age (Fig. 4). Mann-Whitney U analysis also indicated a significantly ( $z$ , corrected for ties = -3.09,  $p < 0.001$ ) greater spat height per hour immersed (HT/HR) for subtidal (mean = 0.029 mm, SE = 0.001) than intertidal oysters (mean = 0.023 mm, SE = 0.001). At  $\geq 850 \text{ HRS}_t$ ,

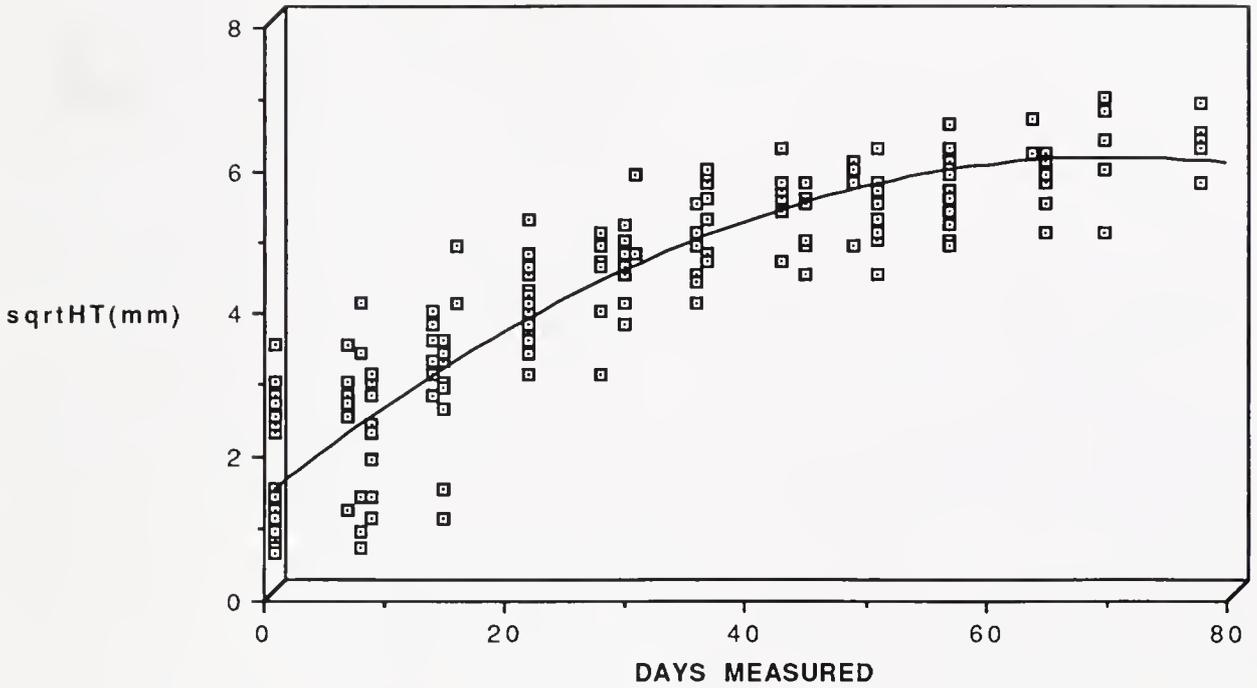


Figure 3. Polynomial regression of square root of subtidal spat height (sqrtHT) as a function of days measured. Regression equation is as follows:  $\text{sqrtHT}(\text{mm}) = 1.39 + (0.13)(\text{days}) - (9.51 \times 10^{-4})(\text{days})^2$ ;  $p = 0.0001$ ;  $r = 0.926$ .

however, HT/HR was no longer significantly different between subtidal and intertidal oysters.

DISCUSSION

Increase of spat height with days measured (Fig. 2a and b) was not an unexpected result of this study as size tends

to be directly related to age for young filter-feeding organisms (Klekowski and Duncan 1975). Size of larval (His and Maurer 1988), juvenile (Morales-Alamo and Mann 1990) and adult (Epifanio and Mootz 1976) oysters have been demonstrated to increase with age. Figure 5 illustrates the theoretical relationship between relative shell heights

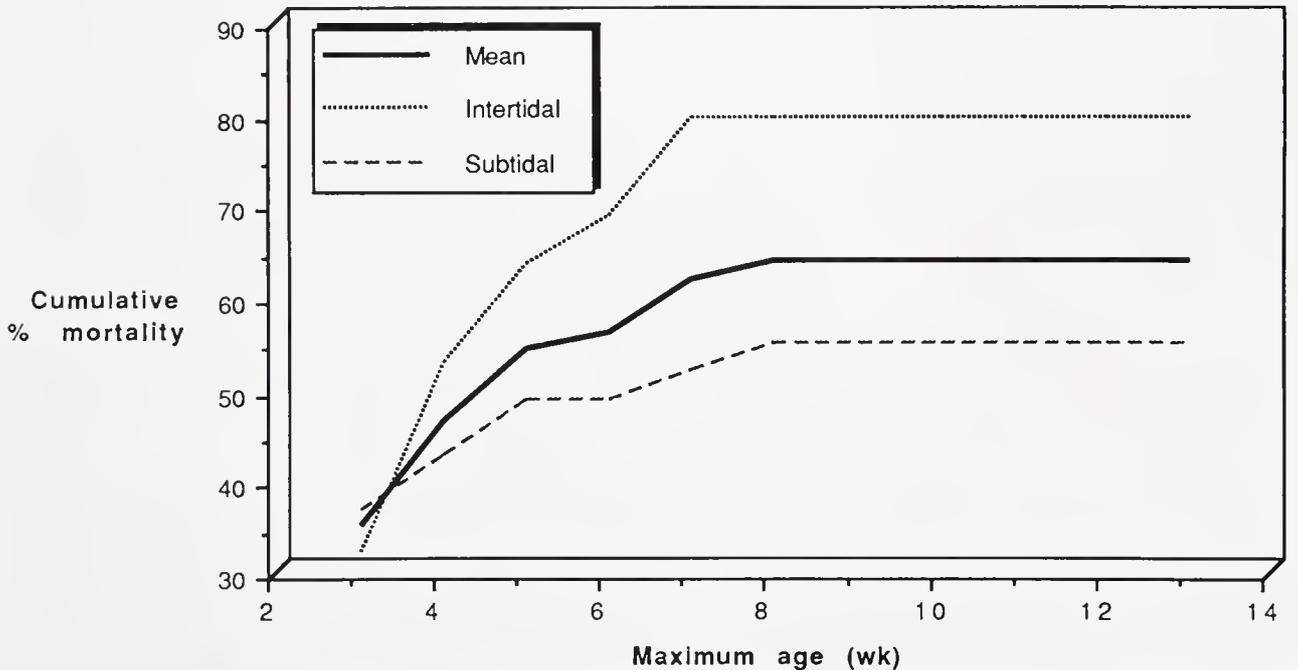


Figure 4. Cumulative % mortality of intertidal and subtidal spat that survived at least two weeks on collecting plates. (See text for details).

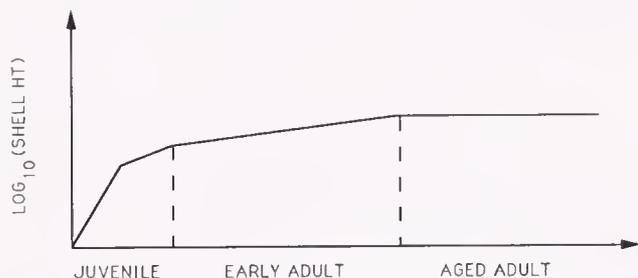


Figure 5. Graphical illustration of the theoretical relationship between shell height and age in bivalves. (See text for details).

and different life stages of post-metamorphosed bivalves. Our study dealt with the period from metamorphosis to mid/late juvenile stage. Although intertidal and subtidal spat height increased at equal rates during this period when linearly regressed with days measured, and mean height of the two spat populations was not significantly different on the initial days of measurement, the intercept of the height with days measured model was significantly greater for subtidal than intertidal spat (Fig. 2a and b). In addition, the overall mean of subtidal spat height was significantly greater than that for intertidal spat. These results are indicative of differential rates of growth within and between intertidal and subtidal oysters, over time. When analyzed together, cumulative growth rate is significantly and positively related with  $HRS_t$ . However, since mean cumulative growth rate is significantly greater for subtidal than intertidal spat, any relationships between cumulative growth rate and other variables must be independently analyzed for the subtidal and intertidal populations. When such independent analyses were carried out, no significant relationship existed for subtidal cumulative growth rate with  $HRS_t$ .

The critical point of differential cumulative growth rate between subtidal and intertidal spat began immediately post-metamorphosis and is likely due, initially, to the greater time of immersion and, thus, greater time for energy acquisition by the subtidal spat. Adult intertidal *Crassostrea gigas* growth has been reported to be negatively related with tidal height (Woelke 1959). This relationship did not remain constant throughout our study, however. Prior to  $\sim 850 HRS_t$ , subtidal spat grew at a significantly greater cumulative and periodic growth rate than intertidal populations, although subtidal spat growth rates did not significantly increase with increasing  $HRS_t$ . The initially lower intertidal cumulative growth rate did significantly increase with  $HRS_t$  until  $\sim 850 HRS_t$ , at which time a) intertidal cumulative growth rate was no longer significantly correlated with  $HRS_t$  and b) periodic growth rate became greater for the intertidal versus subtidal population. Our data indicated that this was due to an increase in intertidal periodic growth rate rather than a decrease in subtidal rates. Floating food particles (i.e., pollen grains) that were not available to the subtidal spat and were initially too large for either spat population to ingest, may have provided a sup-

plemental source of nutrition to intertidal spat as they grew larger. At  $\geq 850 HRS_t$ , subtidal and intertidal cumulative growth rate and square root of height were no longer significantly different. When regressed with days measured at  $\leq 850 HRS_t$ , square root of subtidal spat height had a significantly greater slope than intertidal spat, although the sub- and intertidal intercepts were equal. At  $\geq 850 HRS_t$ , however, the subtidal slope was significantly less, yet the subtidal intercept was significantly greater than the intertidal equation. It is clear (Fig. 3), that the increase in subtidal spat height begins to plateau at  $\sim 55$  days. This corresponds to  $\sim 1320 HRS_t$  for the subtidal spat. The intertidal spat, however, had only been submerged for  $\sim 900 HRS_t$  at 55 days. At 77 days (the final measurement), the intertidal spat had been submerged a similar number of hours and had attained similar size as the subtidal spat had at 55 days.

These results indicate that a) intertidal spat were initially at some selective disadvantage that was manifested in a reduced cumulative growth rate and periodic growth rate, and/or b) subtidal spat were initially at some selective advantage that was manifested in an accelerated cumulative growth rate and periodic growth rate. The fact that with increasing  $HRS_t$ , the intertidal cumulative growth rate increased while the subtidal cumulative growth rate remained constant would indicate that the intertidal spat were, initially, at some selective disadvantage. However, the significantly greater increase in subtidal height with  $HRS_t$  before, versus after,  $850 HRS_t$  would indicate that subtidal spat may also have had a selective advantage in the earlier post-metamorphosis stage. This data is strong evidence that immersion time has a significant influence on oyster spat growth during the early post-metamorphosis stage, and that intertidal spat were able to compensate for an initially lower cumulative growth rate by steadily increasing it over time.

The second order polynomial relationship of square root of height with  $HRS_t$  for intertidal and subtidal spat is graphically illustrated in Figure 6. At  $< 850 HRS_t$ , the intertidal spat almost all fall below the regression line; a manifestation of lower intertidal cumulative growth rate and periodic growth rate. Due to the significant positive relationship between intertidal cumulative growth rate and  $HRS_t$ , however, the intertidal population was able to make-up for their earlier deficient rate of growth and achieve similar size as their subtidal siblings by  $\sim 850 HRS_t$ . One must keep in mind that  $850 HRS_t$  corresponds to  $\sim 52$  days for intertidal and  $\sim 35$  days for subtidal spat.

The question then arises as to how intertidal spat are able to catch up to subtidal spat and why subtidal spat growth rates shift dramatically at  $\sim 1320 HRS_t$ . We hypothesize that oyster spat exhibit a rapid rate of growth in the early/mid juvenile stage which dramatically shifts to a much slower rate of growth at some genetically pre-determined size. The metabolic cause for such a shift may be the initiation of energy reallocation from totally somatic

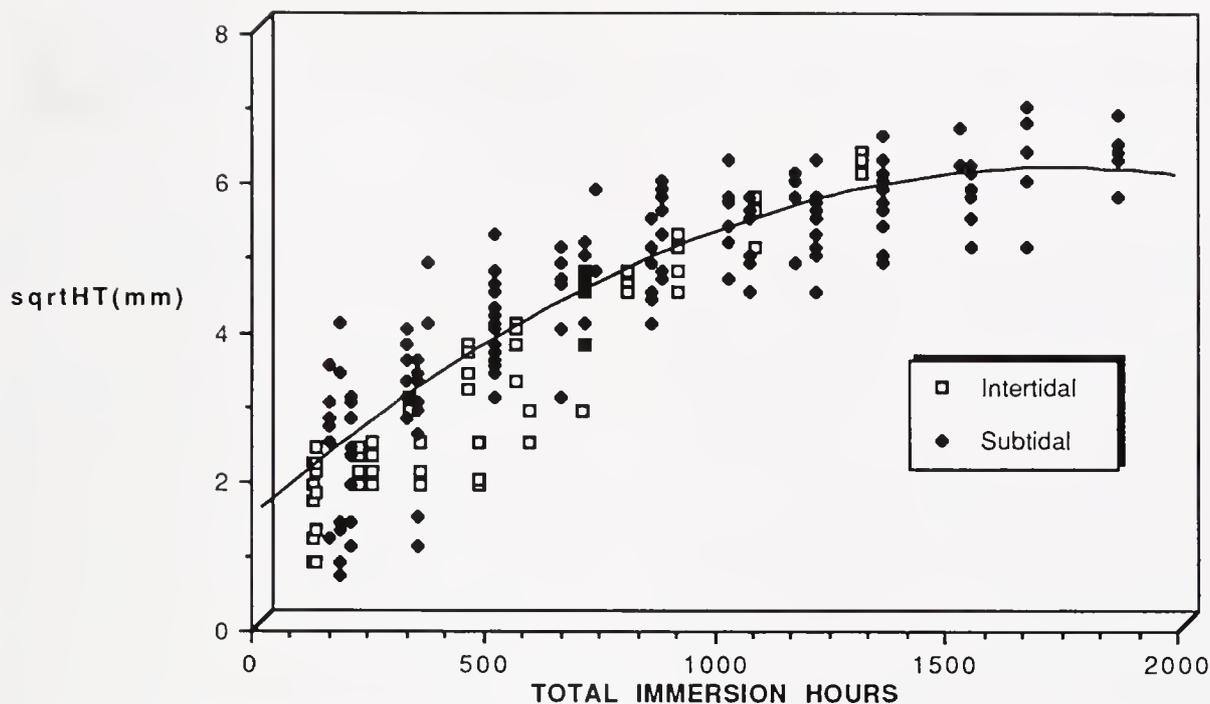


Figure 6. Polynomial regression of square root of shell height for intertidal and subtidal spat as a function of total hours immersed ( $HRS_T$ ). Regression equation is as follows:  $\text{sqrtHT}(\text{mm}) = 1.48 + (5.31 \times 10^{-3})(HRS_T) - (1.53 \times 10^{-6})(HRS_T)^2$ ;  $p = 0.0001$ ;  $r = 0.892$ .

growth to some mixture of gametic and somatic growth. This is not to say that the spat become sexually mature at this time, only that the initiation of maturation processes may begin to occur. We speculate that a more important metabolic factor, however, involves the fluctuation of anabolic processes resulting in a temporary reallocation from principally shell growth to primarily soft tissue growth. Temporal variations in shell and soft tissue growth have been reported for *Geukensia demissa* (Borrero and Hilbish, 1988).

The significant increase in intertidal cumulative growth rate may be due to natural selection for individuals capable of greater assimilation efficiency (AE) than generally found in subtidal spat. The physiological avenue by which an increased AE is obtained may be a) increased production of and/or b) higher rates of activity of digestive enzymes (i.e., cellulases). At low tide, exposed oysters close their shells and stop feeding, resulting in food material remaining in their guts until re-immersion by the rising tide when feeding begins again. It has been suggested (Crosby et al. 1989) that since intertidal oysters have an increased time for enzymatic digestion action to occur on ingested crude fiber, a resulting increased efficiency of utilization of that refractory substrate may occur. This would be dependent on this oyster's ability to carry out aerial digestion and absorption. Intertidal spat lacking the metabolic capacity to compensate for reduced feeding time would be expected to a) have a lower cumulative growth rate and periodic growth rate than those intertidal siblings which did have the com-

pensatory capability and b) have a reduced chance of survival. An earlier study of growth rates in bivalves as a response to immersion time found that although greatly reduced growth was exhibited with increasing percent aerial exposure, the magnitude of the percent reduction in rates was 2–3 times the percent reduction in submergence time (Peterson and Black 1988). Intertidal spat in our study were exposed ~20% of the tidal cycle and would therefore be expected to have a cumulative growth rate that was 80% of subtidal cumulative growth rate (mean =  $0.5 \text{ mm day}^{-1}$ , SE = 0.02), if immersion time alone completely explains growth rates. However, intertidal cumulative growth rate (mean  $0.27 \text{ mm day}^{-1}$ , SE = 0.02) exhibited double the expected decrease and was comparable to what one would predict from the work of Peterson and Black (1988). Therefore, differences in feeding time alone are insufficient to explain completely the reduced intertidal growth observed in our study. Losee (1979) reported that oyster spat which metamorphosed during the first three days of the setting period were significantly larger at 29 weeks post-setting than spat that set later during the setting period. However, in an earlier study Losee (1978) had reported results which contradicted her later study. Regardless, it is highly unlikely that intertidal spat in our study would have set on different days than subtidal spat, due to a) the homogeneous nature of the water column in North Inlet, b) rapid current velocities, c) the intertidal spat plates having only 20% less time for settling to occur than subtidal plates, and d) intertidal and subtidal plates being only 60 cm apart.

Mann-Whitney U analysis of mean maximum age of spat in our study indicated significantly greater mortality in intertidal versus subtidal spat. It is possible that spat mortality observed on our collecting plates may not be representative of mortality in reef conditions where mud and shading may aid spat survival. Relative comparison of intertidal versus subtidal spat mortality observed in our study is, nevertheless, valid due to identical substrate conditions (i.e., both populations were on plates, not reefs). Graphical illustration of mortality (Fig. 4) demonstrates a plateau of intertidal spat mortality ( $\sim 79\%$  of the initial intertidal experimental population) at 7 weeks ( $\sim 810$  HRS<sub>t</sub>). Intertidal spat surviving to this period may have possessed a genetically pre-determined compensatory capability that allowed them to achieve the same cumulative growth rate, for our study period, as subtidal spat by increasing periodic growth rate with HRS<sub>t</sub>. Hence at  $\geq 850$  HRS<sub>t</sub>, no significant difference was observed between subtidal and intertidal spat height or cumulative growth rate. Unfortunately, intertidal spat growth was only measured for  $\sim 1320$  HRS<sub>t</sub> in our study. Had the study been for a longer time period and intertidal periodic growth rate continued to be greater than subtidal periodic growth rate, intertidal oysters may have eventually acquired both greater cumulative growth rate and size. From the eighth week until the end of this study (13 wk), no mortality was observed for either intertidal or subtidal spat. In a review of oyster ecology in the South Atlantic United States, Burrell (1986) found that although heaviest spatfall tended to occur below mean low water, survival is much greater at just above mean low water due to greater subtidal predation and fouling. The studies on which Burrell based his statements dealt with benthic spat. The subtidal spat in our study were suspended, thus would likely have been subjected to much less pressure from benthic predators (i.e., crabs and snails) and have enjoyed greater current velocities resulting in less siltation. It should also be remembered that biofouling was eliminated and the undersides of collecting plates were used in our study. Manzi et al. (1977) compared growth of seed oysters ( $\sim 44$  mm) set subtidally that were replaced to floating and bottom culture. They found significantly greater growth in the floating versus bottom trays. It is quite likely that greater fouling and predation, as well as food limitation in the benthic boundary layer (Frechette and Bourget 1985) prevented the benthic population from achieving as great a growth rate as their suspended siblings. It is unlikely that two populations of spat in our study experienced any significant difference in food quality or quantity  $l^{-1}$  due to their close horizontal positioning ( $\sim 60$  cm) relative to each other and both populations being  $\sim 2$  m above the bottom, as well as the homogeneous nature of the water column discussed earlier.

Gillmor (1982) found that oyster growth was greater per unit immersion time at depths below mean high water in the

intertidal than in subtidal populations. The results of our study would seem, on preliminary observation, to contradict those of Gillmor. His study concluded that oyster growth was best at low intertidal ( $\sim 20\%$  aerial exposure). The intertidal spat in our study were also exposed  $\sim 20\%$  of the tidal cycle yet the data presented here indicate a greater subtidal cumulative growth rate. We also found significantly greater spat growth per unit immersion time in subtidal versus intertidal oysters until each population was immersed for 850 h. At  $\geq 850$  HRS<sub>t</sub>, inter- and subtidal growth per unit immersion time was no longer significantly different. There are, however, major differences between these two studies which warrant discussion. The spat population that we studied settled and underwent metamorphosis in the field and had initial measurements of  $\sim 1.5$  mm. Gillmor's populations were hatchery reared and had initial heights an order of magnitude greater than ours. The water temperatures in our study site were  $\sim 27^\circ\text{C}$  versus  $14^\circ\text{C}$  for the earlier study. While water and air temperatures were very similar for our study, intertidal spat in the earlier study likely experienced a doubling in temperature when aerially exposed, which would in turn increase the activity of digestive enzymes in intertidal spat and possibly yield greater available energy for growth than their colder subtidal siblings. We measured growth essentially from metamorphosis and at weekly intervals, whereas Gillmor's study began measurements on oysters at  $\sim 2$  months of age and at monthly intervals. Hence, instead of contradicting results, our study serves to elucidate the natural selection which occurs in the first 2 months of an intertidal spat existence and which may well have led to metabolic differences which were manifested to yield the results obtained by Gillmor. Perhaps the most significant difference between the two studies was the methods for assessing growth. Gillmor utilized dry meat weight, while we measured shell height. Rather than yielding contradicting results, the different methods of measuring growth in the two studies together lend support to our hypothesis of differential rates of growth for soft tissues and shell in oysters. In order to definitively determine if differential rates for tissue growth exist in oysters and how this rate may fluctuate with age and immersion time, the methods of these two studies need to be combined in a future project.

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## DIFFERENTIAL ELIMINATION OF INDICATOR BACTERIA AND PATHOGENIC *VIBRIO* SP. FROM EASTERN OYSTERS (*CRASSOSTREA VIRGINICA* GMELIN, 1791) IN A COMMERCIAL CONTROLLED PURIFICATION FACILITY IN MAINE†

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**ABSTRACT** The potential for depurating indicator bacteria and pathogenic *Vibrio* sp. from oysters, *Crassostrea virginica* (Gmelin, 1791), was evaluated at a commercial controlled purification (CP) facility in Maine. Oysters from restricted Maine waters were analyzed for total coliforms (TC), fecal coliforms (FC), total vibrios (TV), and pathogenic vibrios (PV = *Vibrio vulnificus*, *V. parahaemolyticus*) before and after 48 hours of CP. The purification plant efficiently removed FC from oyster samples during a 27 week summer–late autumn period that spanned a wide range of temperature and salinity conditions. Depuration was less effective at reducing TC, while TV and PV did not respond to depuration. PV were detected in seven of eleven oyster samples collected between July and October. PV became undetectable from both estuarine water and oysters after October while TV remained detectable until December.

**KEY WORDS:** *Crassostrea virginica*, *Escherichia coli*, *Vibrio* sp., controlled purification, coliforms

### INTRODUCTION

Coastal areas of the U.S. have witnessed incredible population increases and accompanying development pressure in the last 20 years. Pollution problems and conflicts between user groups of coastal waters have had great impacts on the shellfishing industry. Guidelines and standards have been developed for microbial contamination levels that protect the public health, and regulation of shellfish harvesting is based on sanitary surveys and routine monitoring of overlying waters for standard indicator bacteria. However, this process has not been completely effective, as outbreaks of shellfish-related illnesses occur frequently and with regularity (Richards 1985, Rippey 1989). With decreasing area open to shellfishing, activities such as illegal harvesting from polluted, prohibited areas, wet storage of harvested shellfish in polluted waters, and other violations of existing regulations become more problematic (DuPont 1986). The negative publicity that has accompanied recent occurrences of food poisoning from consumption of raw or undercooked shellfish contaminated with microbial pathogens has alarmed consumers and depressed the shellfishing industry.

Commercial harvesting of shellfish from moderately

polluted, restricted areas is permitted when shellfish undergo depuration, or controlled purification (CP). The process of CP theoretically removes pathogenic microorganisms from edible shellfish meats, resulting in decontaminated shellfish that can be marketed for direct consumption, raw or cooked. The final product can be sealed and marketed in ways that give distributors and ultimately the consumer confidence that the oysters have been certified as safe for consumption, similar to the pasteurization programs instituted for milk years ago. In practice, controlled purification has been shown to be both effective (Metcalf et al. 1979, Son and Fleet 1980, Timoney and Abston 1984, Kelly and Dinuzzo 1985), and ineffective (Canzonier 1971, Rowse and Fleet 1984, Power and Collins 1989) in removing different types of microbial contaminants from shellfish.

Besides differences in experimental factors such as design and scale of the test facilities, other factors, including whether shellfish are seeded or naturally contaminated, the physiological condition of the shellfish, and the level of initial microbial contamination, can influence the effectiveness of the CP process. In addition, different microorganisms respond differently to CP. Enteric bacteria such as *Escherichia coli*, fecal coliforms, *Salmonella* sp., etc., are usually effectively purged from shellfish by CP (Barrow and Miller 1969, Son and Fleet 1980, Jones et al. 1991). Other microorganisms such as viruses (Metcalf et al. 1979, Richards 1988, Power and Collins 1989), and the indige-

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nous, estuarine *Vibrio* sp. (Greenberg et al. 1982, Jones et al. 1991) respond inconsistently to CP. Viruses and pathogenic *Vibrio* sp. are presently of great concern because of the continued incidence of serious diseases and fatalities associated with consumption of shellfish contaminated by these organisms (Richards 1988). In the best case, CP facilities that successfully deplete enteric bacterial pathogens, i.e., pathogens typically associated with more common, less serious gastrointestinal diseases, should be as effective in removing pathogenic viruses and vibrios. Comprehensive epidemiological studies are needed to determine the actual health risks associated with consumption of shellfish contaminated with pathogenic vibrios, as well as depurated and relayed shellfish. However, the CP process has great potential as a means of purging shellfish of all microbial pathogens, and more detailed studies are needed to determine what could be modified to enhance elimination of these pathogens.

*Vibrio* sp. are common, natural inhabitants of marine and estuarine environments (Oliver et al. 1983). Pathogenic vibrios are especially common in the warmer waters of the Gulf of Mexico and the southern coastal U.S. (Kelly 1982, Oliver et al. 1982), although they are also routinely detected in Chesapeake Bay, Long Island Sound, and Boston Harbor on the east coast (Oliver et al. 1983) and from southern California to British Columbia on the west coast (Kaysner et al. 1987, Kelly and Dan Stroh 1988). In northern New England, vibrios are not detected in estuarine waters until the water warms during late spring, and the pathogenic vibrios are only detected for about four months from July to October (O'Neill et al. 1990, Jones et al. 1991). Vibrios are sensitive to certain environmental factors, including temperature and salinity conditions (Kelly 1982, Singleton et al. 1982), and can be eliminated from oysters relayed to uncontaminated waters in Maine and NH (Jones and Howell, unpublished results). The purpose of this study was to evaluate the relative effectiveness of an operating, commercial CP facility in Maine to deplete pathogenic *Vibrio* sp. and coliform bacteria, under a range of environmental conditions, from oysters harvested from restricted Maine waters.

## MATERIALS AND METHODS

### Sampling Procedures

Oysters were harvested from the conditionally-restricted waters of the Piscataqua River in Maine during 1989. Water samples were collected from the harvest site at low tide on the same day that oysters were harvested. Temperature and salinity of the harvest waters were measured on site with a thermometer and a calibrated refractometer, respectively. Oyster and water samples were refrigerated and transported to the Jackson Estuarine Laboratory (JEL), Durham, New Hampshire. Water and oyster samples were processed for analysis within two hours of collection. Bac-

teriological analyses were performed on water samples, and both freshly harvested and depurated (48 h CP) oysters from the Spinney Creek Oyster Company (SCOC) CP facility located on Spinney Creek in Eliot, Maine. For further details of the design and operation of this facility, see Howell and Howell (1989).

### Bacteriological Methods

Twelve to sixteen individual shellfish from freshly-harvested and depurated samples of each weekly harvest of shellfish were processed for bacteriological analysis. Shellfish were aseptically shucked and the contents homogenized with equal parts of buffered peptone water. The MPN, multiple tube fermentation method used for the detection of total and fecal coliforms (APHA 1985), was carried through confirmed and completed tests in accordance with recommended procedures. EC tubes that produced gas were considered positive for fecal coliforms, and a portion of these tubes were streaked for colony isolation onto EMB agar. Dark colonies with metallic sheens or other representative colony types were transferred to nutrient agar slants and further analyzed to confirm the presence of *Escherichia coli* using routine IMViCs procedures, oxidase, and Gram stain tests.

Vibrio analysis involved decimal dilution of samples in alkaline peptone water as a three-tube MPN assay. Turbidity dilution broth tubes were streaked onto thiosulfate-citrate-bile-sucrose (TCBS) agar and all different resultant colony types transferred to peptone broth containing 0% and 3% NaCl. Isolates that did not grow in 0% NaCl were further characterized to determine if they were vibrios. Isolates which formed blue-green colonies on TCBS, and thus suspected *V. vulnificus* or *V. parahaemolyticus*, were further characterized using growth in 6%, 8%, and 10% NaCl, ornithine and lysine decarboxylase, arginine dehydrogenase, and cellobiose fermentation tests, the API 20E identification system, and a latex-bound antigen specific for *V. vulnificus*.

## RESULTS

Fecal coliforms were present in freshly-harvested oysters at levels ranging from 50 to 50,000 per 100 g meat (Fig. 1). FC were consistently and efficiently purged from oyster tissue during 48 hour CP processing on each of the 14 sample dates. FC levels ranged from <2 to 46 per 100 g in depurated oysters, well under the market maximum of 230 per 100 g. Target endpoint FC levels were often achieved after only 24 hours of CP (data not shown), although the purification process became less rapid as water temperatures decreased below 10°C. The efficiency of FC removal by CP was most dramatic from July to September, and diminished on an absolute scale during December, when water temperatures dropped to 0°C. However, even during December FC levels were  $\leq 34$  per 100 g in depurated oysters.

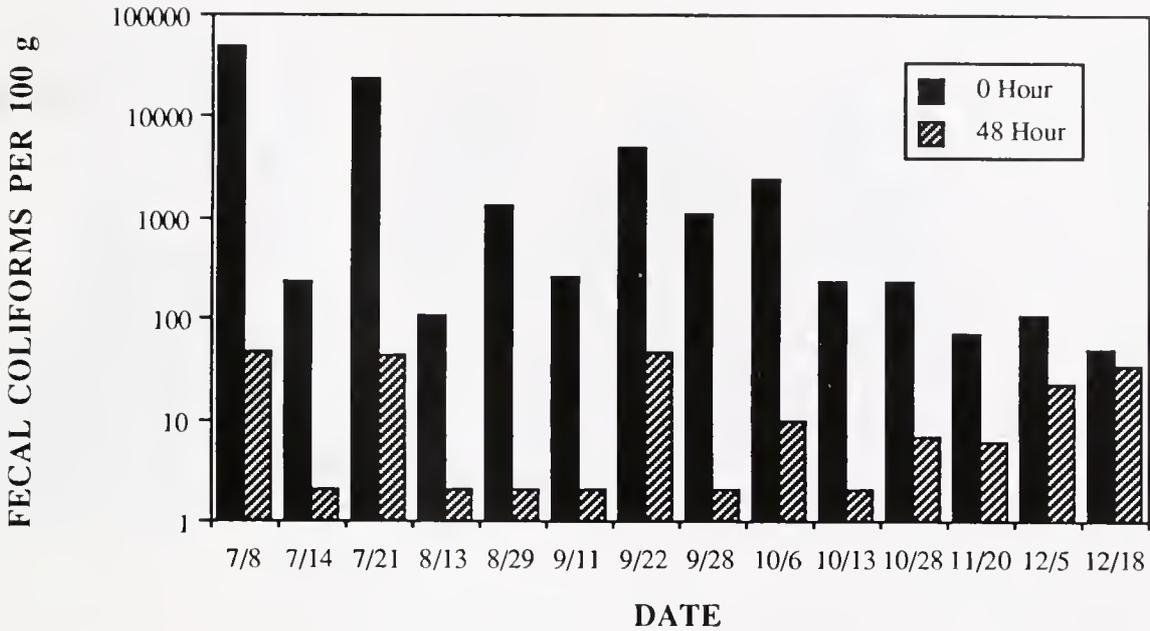


Figure 1. Fecal coliforms in oysters before and after controlled purification.

Total coliforms are a group of bacteria that include many more bacterial species than fecal coliforms (Grimes 1987). TC levels in oysters were greater than FC levels for each sample, yet responded to CP in a fashion similar to the FC response (Fig. 2). TC levels in freshly harvested oysters ranged from 240 to 160,000 per 100 g oyster meat, and decreased in response to CP to between <2 and 1800 per 100 g meat. The efficiency with which CP removed TC from oysters was, like the FC response, most dramatic during July, August, and September, and least efficient in December. However, TC levels increased following 48

hour CP in oysters on December 18 from 900 to 1800 per g meat.

Indigenous, estuarine vibrios are not coincident with fecal-borne bacteria in the estuarine environment, and thus may not be expected to respond to CP like coliforms. Levels of TV were relatively high in oysters throughout the study period, ranging from 230 to >2,400,000 per 100 g freshly harvested oysters (Fig. 3). TV levels remained high in oysters despite decreasing in overlying waters during late autumn, where they became undetectable in December (Table 1). Significant removal of TV from oysters fol-

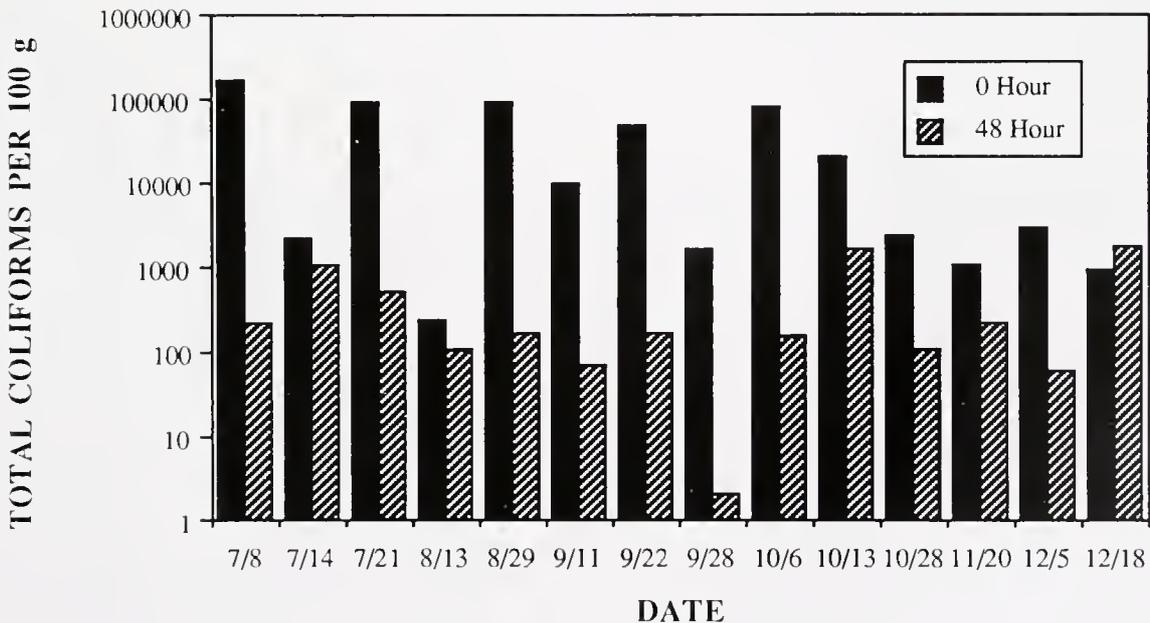


Figure 2. Total coliforms in oysters before and after controlled purification.

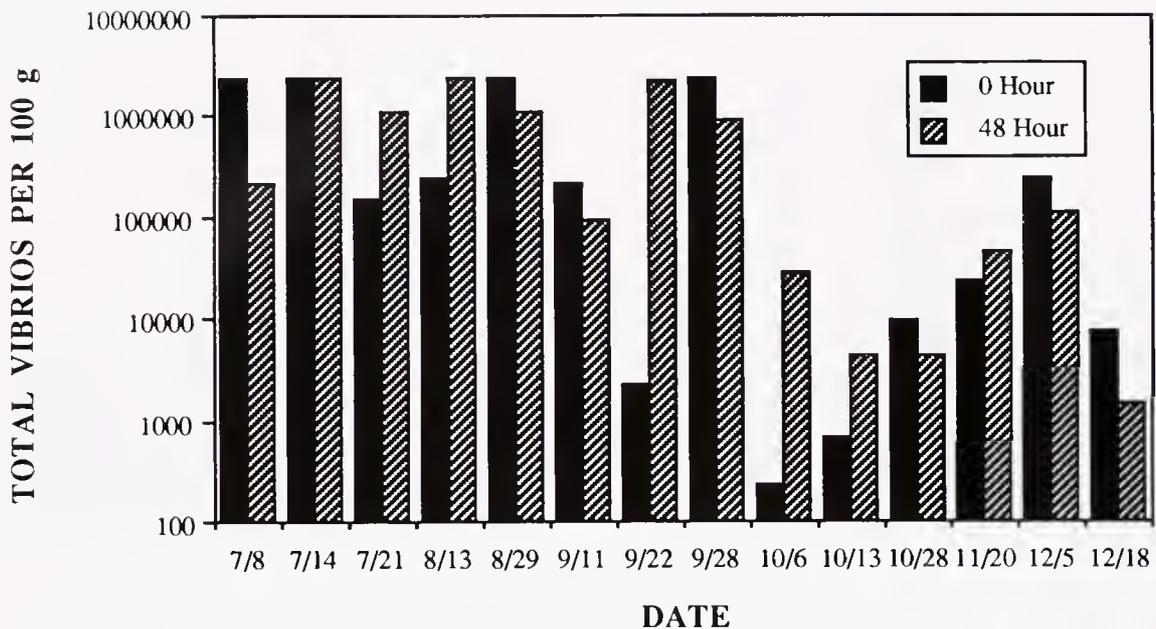


Figure 3. Total vibrios in oysters before and after controlled purification.

lowing CP did not occur in any samples. Significant differences between fresh and processed oysters were apparent on only two dates, when TV levels increased following CP. For example, TV increased from 2300 per 100 g fresh oysters to 2,200,000 per 100 g depurated oysters on September 22, 1989.

Two pathogenic vibrios, *V. vulnificus* and *V. parahaemolyticus*, were also detected in oysters during the last half of 1989. Either one or the other was detected in seven out of the 14 sample dates (Fig. 4). The response of PV to CP was inconsistent, with dramatic decreases in levels on two dates, no detection in fresh oysters followed by detection of significant levels in depurated oysters on two other dates, and essentially no response to CP for samples on the other

dates. PV were not detected in any oyster samples collected before July 8 (Jones et al. 1991), or after October 28, when water temperatures dropped below 12°C. PV were also not detected in any water samples taken from October to December (Table 1). Although temperature is probably the major factor associated with the decline of PV in oysters and water during autumn, low tide salinities in the Piscataqua River were also lower in late autumn ( $\leq 4$  ppt; Table 1) than during summer (average July–September: ca. 17 ppt).

#### DISCUSSION

The process of controlled purification at the Spinney Creek Oyster Company in Eliot, Maine, was effective in eliminating fecal coliform bacteria from oysters harvested over a six month time period. Initial contamination levels in oysters, ranging from 50 to 50,000 FC per 100 g, were consistently reduced to levels below the target level of 20 FC/100 g, and often below detection limits. In relation to other studies, this study is significant because an operating, commercial facility was used for studying CP, the oysters studied were all naturally contaminated, oysters were harvested over a wide range of environmental conditions, samples were tested simultaneously for both vibrios and fecal bacteria, and conditions were carefully maintained to minimize stress (low salinities; spawning; low dissolved oxygen) to the shellfish during the CP process. Use of a commercial CP facility instead of a lab-scale CP system (Timoney and Abston 1984) allows for assessment of how well CP works for shellfish that are actually marketed to consumers. Use of naturally-contaminated shellfish instead of artificially-contaminated shellfish is also a more realistic

TABLE 1.

Low tide temperature and salinity, and concentrations of coliform and vibrio bacteria in the Piscataqua River during autumn, 1989.

Month	Bacterial Type	Average Bacterial Concentration (per 100 ml)	Average Temperature (°C)	Average Salinity (ppt)
October	Total coliform	6000	13	4
	Fecal coliform	1650		
	Total vibrios	121000		
November	Total coliform	9000	4	2
	Fecal coliform	1600		
	Total vibrios	20		
December	Total coliform	20000	0	2
	Fecal coliform	2200		
	Total vibrios	ND†		

† Not detected

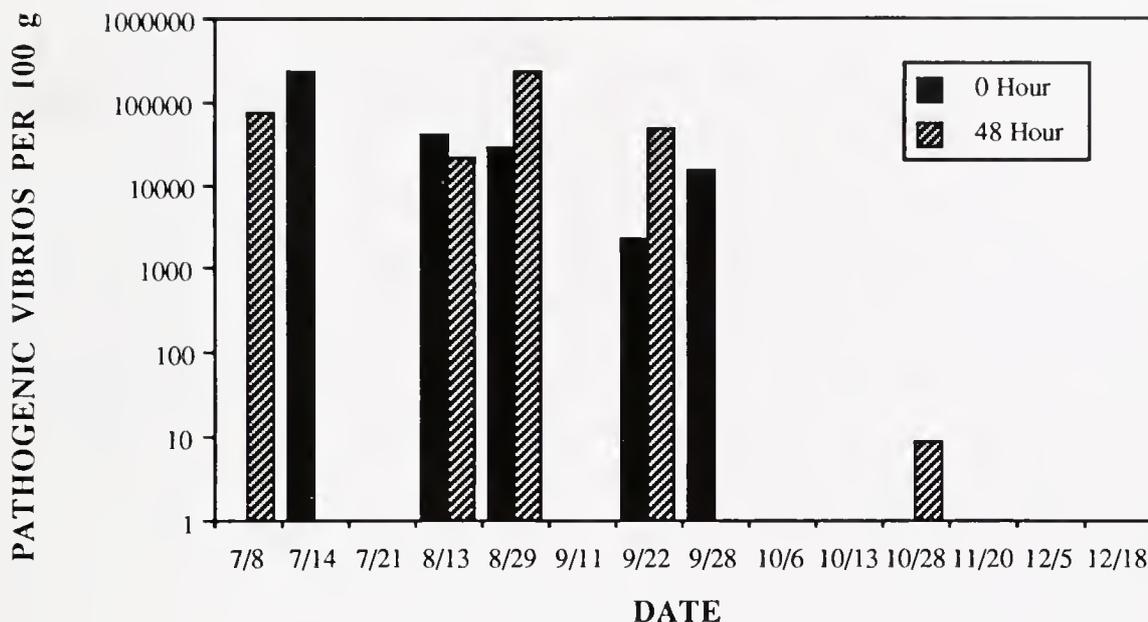


Figure 4. Pathogenic vibrios (*Vibrio vulnificus* and *V. parahaemolyticus*) in oysters before and after controlled purification.

means of assessing potential risks associated with consuming depurated shellfish, especially because laboratory-contaminated oysters may purge contaminants at faster rates than naturally-contaminated shellfish (Heffernan and Cabelli 1971). Simultaneous measurement of fecal bacteria with vibrios allowed for evaluation of the effect of CP on vibrios with confirmation that CP was functioning by causing the effective removal of FC. Healthy oysters are critical for CP studies because stressful conditions can dramatically affect the elimination of microorganisms, especially *E. coli*, from shellfish during controlled purification (Fleet 1978, Power and Collins 1989).

The total coliform test is still accepted by the U.S. FDA (NSSP 1988) and used by the State of New Hampshire as the bacteriological standard for shellfish sanitation. Total coliforms constitute a broad range of different bacteria, and are generally perceived as poor indicators of fecal contamination (Grimes 1987). The response of total coliforms to CP was not as dramatic as the response for FC. In fact, levels of TC apparently increased during CP in one sample. This could be attributed to regrowth of the TC in the shellfish accompanying more effective attachment mechanisms to oyster tissue by some TC and/or sequestering of TC in tissues other than the GI tract which are not responsive to CP. Bacteria isolated as total coliforms from estuarine waters probably include non-fecal, indigenous estuarine bacteria. The differential responses of fecal and total coliforms to CP suggest that the more fecal-specific fecal coliforms may be more easily eliminated from shellfish compared to the total coliforms. Others (Son and Fleet 1980, Vasconcelos and Lee 1972) reported total plate counts for depurated oysters to be around  $10^4$  cells per g, which is consistent with our observations that oysters may retain

some bacteria while selectively eliminating others. These observations have profound public health implications related to depuration of shellfish and the bacteriological indicator used to assess elimination of all microbial pathogens.

*Vibrio* sp. are common, natural inhabitants of marine and estuarine environments (Oliver et al. 1983), including the surfaces and the intestinal contents of marine animals (Baumann et al. 1984). The response to CP by these bacteria, which are constituents of the natural microflora of oysters (Colwell and Liston 1960), may be expected to be different from the response of microbial contaminants of fecal origin. In fact, neither total vibrios (TV) or pathogenic vibrios (PV) were removed from oysters by CP in this study. Vibrios may have evolved survival characteristics that allow them to remain associated with oysters even when the oysters are actively filtering water (Colwell and Liston 1960). This may be advantageous to oysters, as "blooms" of bacteria such as vibrios may serve as important sources of nutrition for oysters and other estuarine filter-feeders during the spring and summer when shellfish are most active (Langdon and Newell 1990). Conversely, it has also been suggested that elevated levels of vibrios may be associated with oyster mortality in shellfish finishing systems (Raymond 1990).

The lack of response to CP by vibrios may be caused by their ability to remain attached to oyster tissue under CP conditions, to colonize oyster tissue previously inhabited by fecal-borne contaminants, or to simply grow at rates that exceed their depletion rates. Greenberg et al. (1982) suggested that vibrios may persist in depurated clams because of their close association with clam tissue and their ability to multiply and colonize clam tissues as other organisms are eliminated. We have found that *V. vulnificus* can be

eliminated from oysters that have been relayed to uncontaminated waters both in southern Maine and in New Hampshire (Jones and Howell unpublished results). Factors that may enhance removal of vibrios from shellfish should be investigated. Raymond (1990) suggested that higher salinities could be used in the processing of shellfish to remove vibrios. Suggestions such as depurating shellfish longer than 48 hours to remove vibrios (Kelly and Dinuzzo 1985, Greenberg et al. 1982) must be weighed against the likelihood that the palatability and quality of shellfish will be reduced upon exposure to extended periods of CP, and the negative economic factors associated with delayed marketing.

The public health significance of the lack of reduction in pathogenic vibrio levels in depurated oysters is difficult to assess, as shellfish-borne disease incidence often goes unreported, may not be linked to specific foods or sources of the food, and the epidemiological studies needed to determine risks have not been done (Richards 1988). Pathogenic *Vibrio* sp. are a concern because they can multiply during commercial handling operations (Son and Fleet 1980, Cook and Ruple 1989), and because of disease incidence associated with consumption, generally by immunocompromised individuals, of PV-contaminated shellfish in the southern U.S. (Tacket et al. 1984, Johnston et al. 1985, Rippey 1989). However, very little is known about the epidemiology or infective dose of *V. vulnificus* for immunocompromised or uncompromised individuals (Janda et al. 1988), and there have been no reported cases of *V. vulnificus* infections in northern New England (Rippey 1989).

In the late autumn, water temperatures in the Great Bay Estuary, NH, decline to below 0°C, causing a significant reduction in the physiological activities of shellfish and the disappearance of culturable vibrios. Problems associated with the use of traditional culture techniques for detecting vibrios aside, the inability to detect vibrios in cold oysters may reflect their inability to survive at low temperatures, as suggested by Oliver (1981). Potentially, depuration at temperatures low enough to kill vibrios yet high enough to allow for maintenance of shellfish metabolic activity could be exploited for removing vibrios from shellfish. However, vibrios can persist for extended periods of time in shellfish stored at low temperatures (Cook and Ruple 1989, Kaysner et al. 1989). Others have resuscitated culturable vibrios in shellfish, harvested from cold water, that are exposed to warmer water for 24 h (Tamplin et al. 1990, O'Neill and Jones, unpublished). Further work is needed to determine

what happens to vibrio populations in estuaries of the northeastern U.S. during winter months.

The successful removal of fecal coliforms from shellfish during CP, as required by law, is fortunate, yet cannot be used as evidence for removal of other potential pathogens such as pathogenic vibrios. Development of better standards for shellfish sanitation or improved detection methods for specific pathogens is needed to provide the means to detect the presence of all pathogens of concern. The inconsistent detection of PV during summer months in this study illustrates the shortcomings of existing, culture-requiring methods for detecting these bacteria. Vibrios and pathogens of fecal origin have been shown to exhibit a response to unfavorable environmental conditions that results in the inability to culture the organisms, even though they may remain viable and pathogenic (Xu et al. 1982). Thus, actual levels of any of these bacteria may be completely different, especially in cold water (Oliver 1981, Linder and Oliver 1989) than what is measured by traditional culture techniques. Modifications of gene probe (Wright et al. 1985, Morris et al. 1987, Gerba et al. 1989) and polymerase chain reaction (Bej et al. 1990) techniques are needed that will allow rapid, safe, sensitive, and inexpensive detection of bacterial and viral pathogens without requiring culture of the target organism.

Controlled purification has been proven to be effective for eliminating bacterial enteric pathogens from shellfish and improving the quality of a valuable food resource. Further investigation is necessary to determine if the CP process can be modified for elimination of more recalcitrant viruses and non-enteric bacterial pathogens like pathogenic vibrios. Development of better detection methods for these pathogens is a crucial need for studying this problem. Ultimately, studies that implement these improved detection methods will be needed to determine the public health significance of the incidence of pathogenic vibrios and viruses in shellfish and what impact modified CP and/or relaying can have on minimizing the potential health risks determined from these studies.

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## DOMOIC ACID IN SHELLFISH AND PLANKTON FROM THE BAY OF FUNDY, NEW BRUNSWICK, CANADA

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**ABSTRACT** Mussels and clams sampled from Brandy Cove and Chamcook Harbour in the Bay of Fundy, New Brunswick during August–October, 1988 had domoic acid concentrations from 1.2–38.0 µg/g wet weight of whole tissue. 96.5% of the domoic acid in the mussels was in the digestive gland. Domoic acid was not detected in shellfish from Deadmans Harbour in the Bay of Fundy during the above period or from all three sites during the summer-fall of 1989. When domoic acid was detectable in shellfish, domoic acid was detectable in plankton tows (0.8–3.5 µg/g wet weight), and the dominant species was *Nitzschia pseudodelicatissima*.

**KEY WORDS:** domoic acid, *Nitzschia pseudodelicatissima*, *Mytilus edulis*, mussels, *Mya arenaria*, clams

### INTRODUCTION

In November 1987, a serious outbreak of food poisoning occurred in eastern Canada. The symptoms observed in the patients included nausea, vomiting, and diarrhea. In some cases, particularly elderly patients, these were followed by neurological effects including confusion, disorientation, and loss of short-term memory (Addison and Stewart 1989). Epidemiological studies implicated the consumption of mussels cultured in Cardigan Bay, Prince Edward Island (Fig. 1). Amnesic shellfish poisoning (ASP) was proposed as a name for this illness. During routine mouse bioassay of these mussels for paralytic shellfish toxins (PSP), neurological symptoms and mechanisms of lethality observed differed from those associated with PSP. The symptomology of ASP in mice has been described (Bose et al. 1989).

In mid-December 1987, the toxin in the mussels was identified as domoic acid (Wright et al. 1989). Domoic acid is a potent neuroexcitatory amino acid. The neurological and toxic effects of domoic acid are due to its agonistic action at kainic acid receptors located in specific areas of the brain (Debonnel et al. 1989a; Debonnel et al. 1989b). Subsequently, 107 cases of ASP, including three deaths (possibly five), have been confirmed (Todd 1990).

The pennate diatom, *Nitzschia pungens* forma *multi-series*, was the dominant plankton in Cardigan Bay water sampled in December 1987 (Bates et al. 1989) and produces domoic acid in culture (Subba Rao et al. 1988) in quantities/cell commensurate with those found in blooms. A much less abundant pennate species, *Amphora coffeaeformis*, that was isolated from toxic mussels sampled during the 1987 incident, also produced domoic acid (Maranda et al. 1990).

During late summer to early winter of 1988, domoic acid was detected in shellfish sampled from the Bay of

Fundy in New Brunswick and Nova Scotia, and Cardigan Bay in Prince Edward Island (Gilgan et al. 1990). Several areas of the Bay of Fundy were closed to shellfish harvesting as the concentration of domoic acid in mussels exceeded the acceptable limit (20 µg/g wet weight) initially established by the Department of Health and Welfare.

The objective of this study was to identify the most likely plankton species producing domoic acid in the Bay of Fundy, and to increase the predictability of the occurrence of domoic acid in shellfish. We attempted to follow the kinetics of the production of domoic acid in plankton from the surface layer and the accumulation and distribution of domoic acid in mussels, *Mytilus edulis*, from the Bay of Fundy in southwestern New Brunswick.

### METHODS

In April of 1988 and 1989, blue mussels, *Mytilus edulis*, were purchased from Corkum's Island mussel farm, Nova Scotia. The socked mussels (cultured) were suspended (maximum depth = 3 m) from longlines in Brandy Cove and Deadmans Harbour (Fig. 1). Wild blue mussels were sampled from the intertidal zone in Brandy Cove and Chamcook Harbour. Soft-shell clams, *Mya arenaria*, were also sampled from Chamcook Harbour. Frozen blue mussels, soft-shell clams, and scallop, *Placopecten magellanicus*, were received from the Cardigan River area, Prince Edward Island through the Department of Fisheries and Oceans, Inspection Branch, during December 1987. The shellfish from the Cardigan River area were stored at –15°C until July 1988, prepared for HPLC analysis, stored at –80°C, and analyzed in March 1989.

The shellfish were shucked, drained for 5 min, and homogenized using a Brinkman Polytron homogenizer. An

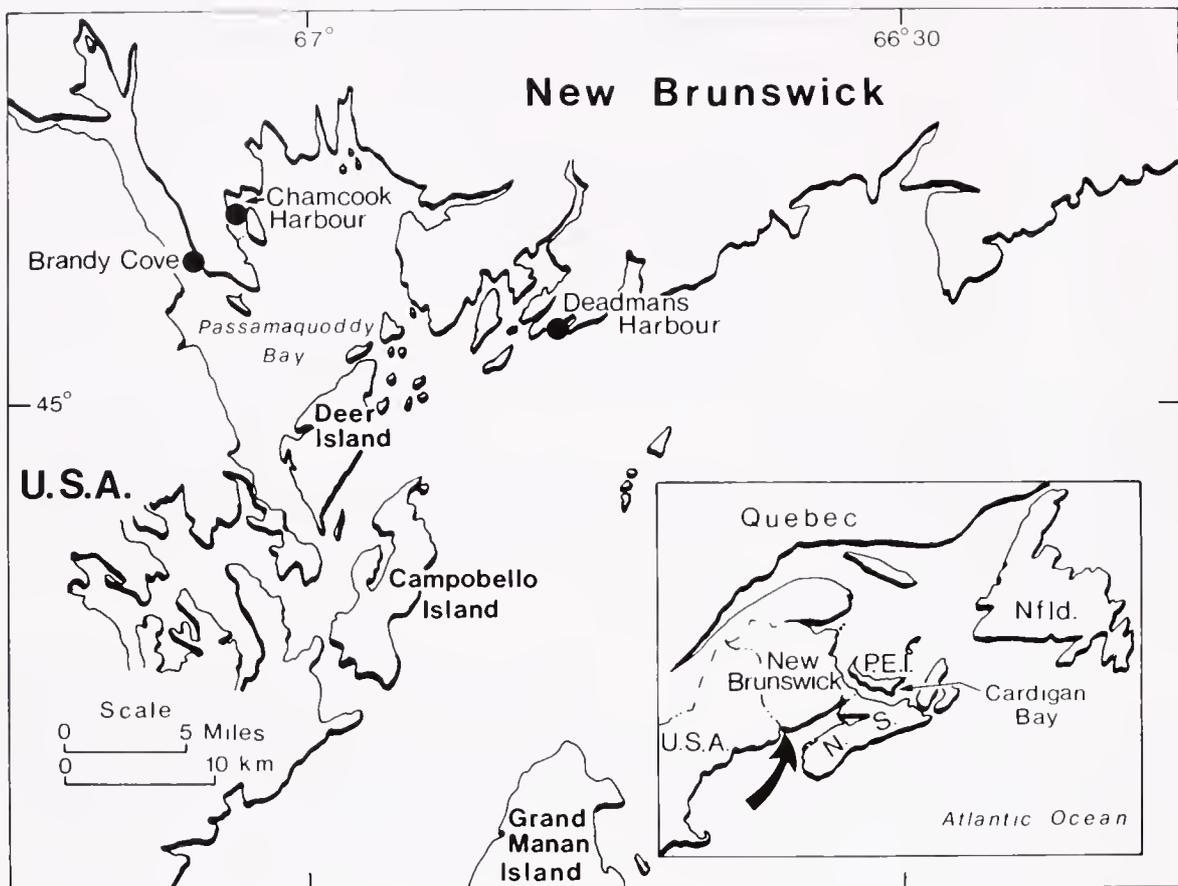


Figure 1. Shellfish and plankton sampling sites in the Bay of Fundy, New Brunswick, Canada.

aliquot of the homogenate (20 g) was mixed with an equal volume of distilled water, boiled for 5 min, cooled, and centrifuged at  $3000 \times G$  for 15 min in an IEC-B20A refrigerated centrifuge. Duplicate aliquots of the supernatant was passed through a Centrifree™ 10 micropartition system (30,000 MW cut-off; Amicon) by centrifugation at  $2500 \times G$  for 60 min. The domoic acid concentration in the filtrate was determined by HPLC. A minimum of 100 g of drained tissue was homogenized. For the determination of domoic acid distribution in mussels, 200 mussels were dissected into foot, mantle, gill, gonad, digestive gland, and the remaining tissue (this included adductor muscle and varying amounts of the tissues above that could not be dissected cleanly).

Phytoplankton were collected with a 20-micron Nitex net ( $\frac{1}{2}$  m diameter) towed at less than 1 knot at the surface. Tow contents were kept on ice during the 1- to 2-h trip to the laboratory. Seawater was removed by aspiration and centrifugation ( $750 \times G$ ; 15 min). The plankton was weighed and resuspended in a minimum amount of distilled water. The slurry was sonicated with a Sonifier™ cell disruptor (Branson) until microscopic examination indicated 90% of the cells were ruptured. Samples were centrifuged and ultrafiltered as above. Domoic acid concentration in the filtrate was determined by HPLC.

Surface water was collected by bucket for determination of plankton abundance. A 200-mL sample was immediately preserved by addition of either 5 mL formalin:acetic acid (1:1) or 2 mL Lugol's iodine solution. A 50-mL aliquot was settled in a settling chamber and the plankton were counted with an inverted microscope.

Domoic acid concentrations were determined by the HPLC method of Lawrence et al. (1989) with the following conditions: Whatman Partisil II ODS-10  $250 \times 4.6$  mm C18 column; Altex Model 110A pump delivering 12.5% acetonitrile in distilled water (pH adjusted to 3 with  $H_3PO_4$ ) at 1 mL/min; 20  $\mu$ L Altex injection loop; UV detection using a Schoeffel SF 770 variable wavelength detector set at 242 nm. Detector response was captured by an Apple IIe computer running Chromatochart™ (Interactive Microwave Inc.). The effluent was fed into a flow-through cell of an HP 8452 diode array UV-VIS spectrophotometer, set to scan between 200 and 300 nm. The diode array data were collected on a Vectra computer running HP software. The calibration curve (20  $\mu$ L injection, 0.4–9.5  $\mu$ g/mL) was prepared with domoic acid (Diagnostic Chemicals, Charlottetown, Prince Edward Island), which was standardized with aliquots of an instrument calibration solution, DACS-1 (89  $\mu$ g/mL; National Research Council of Canada, Halifax, Nova Scotia) diluted with distilled water.

## RESULTS AND DISCUSSION

Confirmation of domoic acid was by coincident retention time on the HPLC and by UV spectrum of the HPLC effluent. Under the HPLC conditions used in the present study (see Methods), domoic acid eluted as a symmetrical peak with a retention time between 11.8–12.2 min (Fig. 2). The detection limit for quantitative analysis of domoic acid was 0.008  $\mu\text{g}$  in a 20- $\mu\text{L}$  injection. Typical chromatograms for mussel and plankton extracts are shown in Figure 2. The mussel extracts contained an unknown compound which eluted just prior to the domoic acid (probably tryptophan; under our conditions  $R_t = 9.8$  min; (Wright et al. 1989)), but it did not significantly interfere with the quantitative analysis of domoic acid.

Examples of UV spectra obtained are shown in Figure 3.

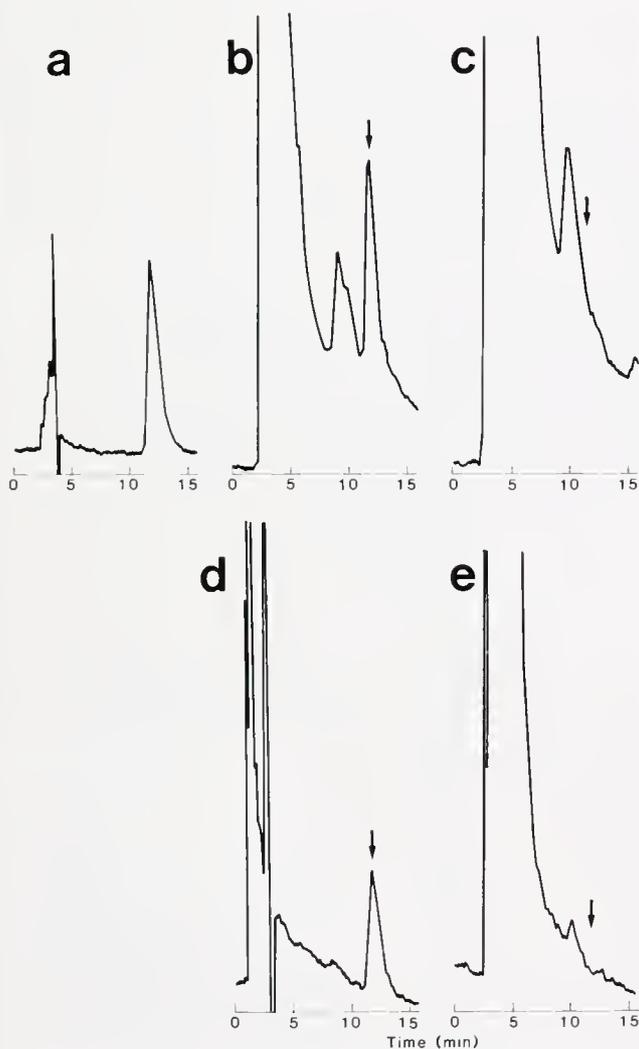


Figure 2. HPLC chromatograms of: a) domoic acid standard (3.5  $\mu\text{g}/\text{mL}$ ); b) and c) digestive gland extracts of Brandy Cove mussels, domoic acid concentration of 4.4  $\mu\text{g}/\text{mL}$  and not detectable, respectively; d) and e) Chamcook Harbour plankton tow extracts, domoic acid concentration of 2.8  $\mu\text{g}/\text{mL}$  and not detectable, respectively. Conditions as described in Methods, full scale absorbance = 0.01 absorbance units.

The detection limit for obtaining the spectra was 0.04  $\mu\text{g}$  of domoic acid in the 20  $\mu\text{L}$  injected into the HPLC, i.e., for concentrations of domoic acid  $>4$   $\mu\text{g}/\text{g}$  wet weight of mussel. For plankton extracts, spectra could be obtained for concentrations  $>2$   $\mu\text{g}$  of domoic acid/g wet weight of plankton. Greater sensitivity was obtainable with plankton extracts compared to mussel extracts because, with the former, there were fewer co-elutants. At concentrations of domoic acid below 4  $\mu\text{g}/\text{g}$  wet weight of mussel, the domoic acid peak was obscured. Some interference is evident in the UV scan of the HPLC peak corresponding to domoic acid in a mussel extract shown in Fig. 3.

The concentrations of domoic acid found in shellfish from our sampling sites (Fig. 1) during the summer of 1988 are given in Table 1. No domoic acid was found in shellfish from Deadmans Harbour in 1988 or from all sites during summer and fall of 1989. In Chamcook Harbour, the highest concentration of domoic acid observed was 38  $\mu\text{g}/\text{g}$  wet weight of whole mussel. These were wild mussels sampled from the intertidal zone on September 7, 1988. Similar concentrations were reported by Gilgan et al. (1990) for mussels from the same area (Bar Road). Concentrations of domoic acid in cultured mussels (suspended from longlines, therefore constantly submerged in seawater) from Brandy Cove probably peaked around September 19, 1988, based on the concentrations (maximum of 160  $\mu\text{g}/\text{g}$  wet weight) found in the digestive gland. Domoic acid was also found in clams sampled from Chamcook Harbour in 1988, but at lower concentrations in whole tissue (2.6  $\mu\text{g}/\text{g}$  wet weight) than in the wild mussels (26  $\mu\text{g}/\text{g}$  wet weight) from the same area and day (September 14). However, by September 26, the concentrations of domoic acid in the digestive glands of the two shellfish were not significantly different.

In mussels sampled from Brandy Cove on September 19, most of the domoic acid (96.5%) was present in the digestive gland (Table 2). Small amounts were found in the gills (1.1%) and remainder (2.8%; some may be due to digestive gland tissue that could not be completely separated from other tissues). Domoic acid was not detected in the mantle, foot, or gonad.

The concentrations of domoic acid found in shellfish sampled from Prince Edward Island during the winter of 1987/88 were considerably higher than in shellfish from the Bay of Fundy in 1988 (Table 3). The highest concentration of domoic acid (345  $\mu\text{g}/\text{g}$  wet weight) was found in whole mussel tissues originating in produce returned from commercial outlets on December 4, 1987. Domoic acid concentrations were even higher in the digestive gland extracts than in the extracts of whole mussel tissues. The highest concentration found in the digestive gland was 428  $\mu\text{g}/\text{g}$  wet weight. By February 1988, domoic acid was not detectable in the cultured mussels. Wild mussels sampled 1 km upstream from the mussel culture site had lower concentrations of domoic acid than cultured mussels sampled on the same day (50 and 262  $\mu\text{g}/\text{g}$  wet weight, respec-

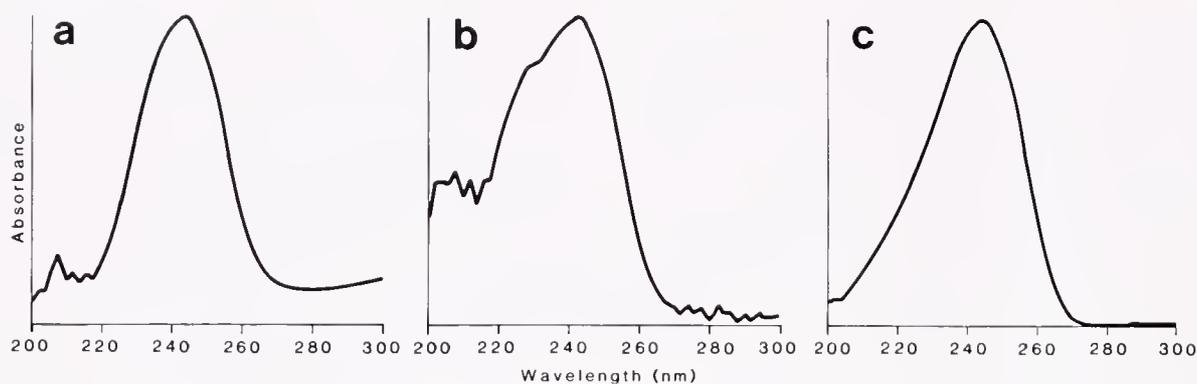


Figure 3. UV spectrum of HPLC effluent corresponding to the domoic acid peak for: a) domoic acid standard; b) Brandy Cove mussel digestive gland extract; and c) Chamcook Harbour plankton extract. Conditions as described in Methods, full scale absorbance = 0.007, 0.007 and 0.070 absorbance units for a), b) and c), respectively.

tively). The concentrations of domoic acid in our samples were comparable to those reported by other investigators for shellfish from Prince Edward Island (Lawrence et al. 1989, Wright et al. 1989). Extremely high concentrations of domoic acid (4180  $\mu\text{g/g}$  wet weight) were found in the digestive gland of some Prince Edward Island scallops (Table 3).

The dominant plankton species in the surface water

whenever domoic acid was detected in shellfish from the Bay of Fundy was *Nitzschia pseudodelicatissima* (Table 1) (Martin et al. 1990). Plankton tows from Brandy Cove and Chamcook Harbour obtained when domoic acid was detected in shellfish also had measurable levels of domoic acid (Table 4). The highest concentration of domoic acid observed in plankton tows was 3.5  $\mu\text{g/g}$  wet weight.

There was no direct quantitative relationship between

TABLE 1.

Domoic acid concentrations in shellfish sampled from Bay of Fundy in southwest New Brunswick, Canada, in the summer of 1988.

Sample	Date	Domoic Acid ( $\mu\text{g/g}$ wet wt)		Dominant Species
		Whole	Digestive Gland	
Brandy Cove				
CM	July 20	ND	ND	NP
CM	Aug 15	1.6	3.0	NP
CM	Sept 14	1.2	17.6	NP
WM	Sept 14	NA	26.0	NP
CM	Sept 19	NA	160.0	NP
CM	Sept 26	NA	6.5	NP
CM	Oct 17	ND	ND	<i>Mesodinium rubrum</i>
Chamcook Harbour				
WM	Sept 1	30.8	NA	NP
WM	Sept 7	38.0	NA	NP
WM	Sept 14	26.0	NA	NP
WM	Sept 26	NA	90.0	NP
WM	Oct 4	NA	6.0	NP
WM	Oct 11	ND	ND	<i>Scrippsiella trochoidea</i>
C	Sept 14	2.6	10.4	NP
C	Sept 26	NA	80.0	NP
Deadmans Harbour				
CM	July 19	ND	ND	<i>Alexandrium fundyense</i>
CM	Aug 22	ND	ND	<i>Peridinium</i> sp.
CM	Sept 19	ND	ND	NP
CM	Oct 17	ND	ND	<i>Mesodinium rubrum</i>

ND = not detected, <0.4  $\mu\text{g/g}$  wet wt.

NA = not analyzed.

NP = *Nitzschia pseudodelicatissima*.

CM = cultured mussels.

WM = wild mussels.

C = clams.

TABLE 2.

Domoic acid concentrations in tissues of mussels cultured in Brandy Cove, New Brunswick, Canada, and sampled on September 19, 1988.

Tissue	Concentration (µg/g wet wt)	Amount (µg) per Mussel	% of Total
Digestive gland	160.0	137.0	96.5
Gill	4.8	3.4	2.4
Gonad	ND	ND	ND
Mantle	ND	ND	ND
Foot	ND	ND	ND
Remainder	2.8	1.5	1.1

ND = not detected, <0.4 µg/g wet wt.

cell density of *N. pseudodelicatissima* in surface waters and the concentration of domoic acid in plankton tows. This is because the cell densities in plankton tows were not related to cell densities in surface waters. Variables that were not determined included: length of time of tow; speed at which the tow was taken; patchiness of plankton blooms; cell breakage and leakage of domoic acid during the tow; differences in production rates of domoic acid depending on the stage of the *N. pseudodelicatissima* bloom. However, the data indicate that when the cell density in surface samples was  $10^5$  cells/L or greater, domoic acid could be detected in plankton tows. Domoic acid was not detected in similar plankton tows during late 1988 or in the summer and fall of 1989. The highest cell densities of *N. pseudodelicatissima* observed during 1989 in surface water at Deadmans Harbour, Chamcook Harbour, and Brandy Cove were 42,000 (July 4), 1700 (July 4), and 140 (April 18) cells/L, respectively.

The abundance of *N. pseudodelicatissima* in surface water from our sampling sites during the summer of 1988 is shown in Figure 4. Increased cell numbers of *N. pseudode-*

TABLE 3.

Domoic acid concentrations in mussels and scallop sampled from Cardigan River, Prince Edward Island, Canada.

Sample	Date	Domoic Acid (µg/g wet wt)	
		Whole	Digestive Gland
Scallop	Dec. 11, 1987	380	4180
	Dec. 15, 1987	460	2600
Cultured mussels	Dec. 4, 1987*	345	NA
	Dec. 7, 1987	195	NA
	Dec. 8, 1987	262	324
	Dec. 11, 1987*	234	253
	Dec. 12, 1987	145	428
	Jan. 25, 1988	21	23
Wild mussels	Feb. 8, 1988	ND	14
	Dec. 8, 1987	50	187

ND = not detected, <0.4 µg/g wet wt.

NA = not analyzed.

\* Mussels that were recalled from commercial outlets.

TABLE 4.

Domoic acid concentrations in phytoplankton sampled from the surface in Brandy Cove, Chamcook Harbour, and Deadmans Harbour, New Brunswick, Canada, during the summer of 1988.

Site	Domoic Acid in Plankton (tow)		<i>N. pseudodelicatissima</i> in Surface Water		% of Total Cells/L
	µg/g Wet Wt	Date	Cells/L × 10 <sup>3</sup>	Date	
Brandy Cove	Trace	Aug. 8	31	Aug. 9	49
	0.8	Sept. 20	355	Sept. 21	99
	ND	Oct. 4	1	Oct. 4	30
Chamcook Harbour	2.6	Sept. 23	1201	Sept. 23	99
	3.5	Sept. 30	380	Sept. 30	99
	ND	Oct. 7	57	Oct. 4	96
Deadmans Harbour	ND	Aug. 4	1*	Aug. 9	3

ND = not detected, <0.4 µg/g wet wt.

\* Dominant species was *Mesodinium rubrum*.

*licatissima* were observed in early July in Brandy Cove and Deadmans Harbour, and peaked in September. The highest concentrations of *N. pseudodelicatissima* observed in surface waters at Chamcook Harbour, Brandy Cove, and Deadmans Harbour were  $1.2 \times 10^6$  (September 21),  $3.6 \times 10^5$  (September 21), and  $1.4 \times 10^4$  (September 13), respectively.

Domoic acid was detected in shellfish and plankton from Brandy Cove and Chamcook Harbour only when *N. pseudodelicatissima* was the dominant species (i.e. >90% of all cells) and when there were more than  $10^5$  cells/L. In October of 1988, when concentration of *N. pseudodelicatissima* in the surface water declined, domoic acid became nondetectable. This would suggest that *N. pseudodelicatissima* was associated, in an as yet unknown way, with the production of domoic acid in the Bay of Fundy. Additional evidence to support this contention was obtained when unialgal cultures prepared from *N. pseudodelicatissima* collected locally produced domoic acid (Martin et al. 1990). To date, two species of *Nitzschia* as well as other algae have been associated with the production of domoic acid. *Nitzschia* sp. are ubiquitous. Our studies also indicated that shellfish accumulate and depurate domoic acid readily. Therefore, densities of *Nitzschia* sp. may be developed into an early warning monitor of domoic acid in shellfish. However, before implementation of this potential indicator, more information is required. For example, why is domoic acid found in plankton tows of predominantly *N. pseudodelicatissima* from some areas of the Bay of Fundy but not in those from other areas (Martin et al. unpubl.)? Are there biochemical differences between morphologically identical strains of *Nitzschia* sp.? Are bacteria or other microorganisms involved (cultures of *Nitzschia* sp. that have produced domoic acid have not been axenic)? Are these specific nutrients and other physical and chemical factors that are required for the production of domoic acid?

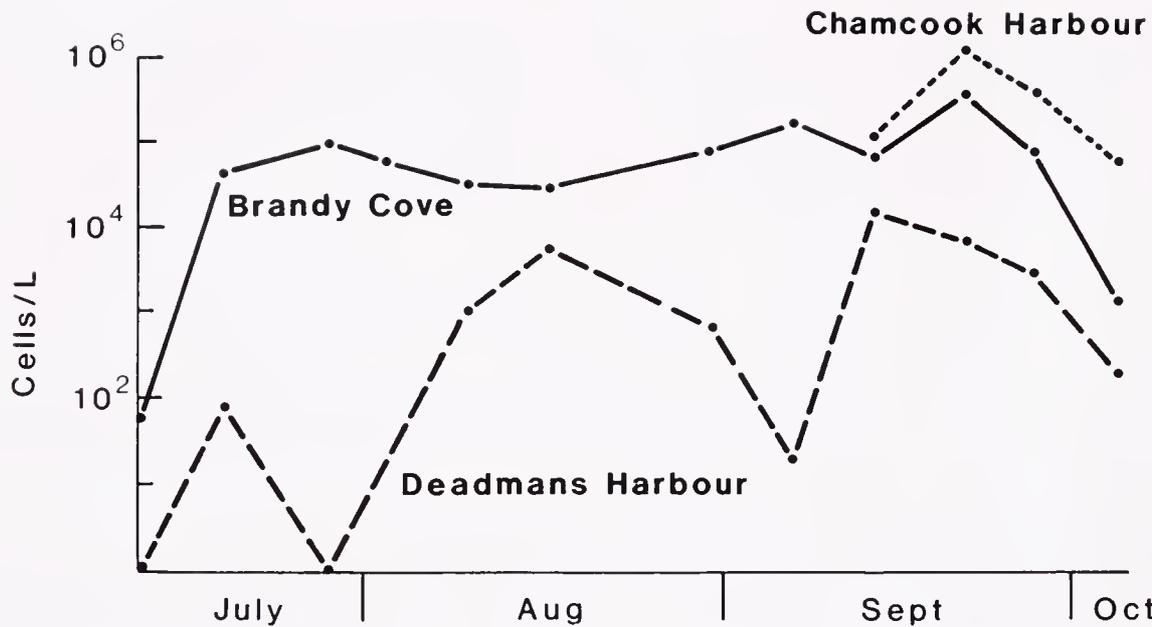


Figure 4. Abundance of *Nitzschia pseudodelicatissima* in surface water samples from Brandy Cove, Deadmans Harbour, and Chamcook Harbour during the summer of 1988.

The occurrence of domoic acid in shellfish is probably not new; we have simply become aware of it. For example, *N. pseudodelicatissima* has been observed in the Bay of Fundy since 1976 (Martin, unpubl.) and by others in the 1930's (Martin et al. 1990). It is probable that domoic acid will be seen as a problem similar to PSP toxins—hazardous and worldwide in occurrence, but manageable.

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## CALIFORNIA'S PARALYTIC SHELLFISH POISONING PREVENTION PROGRAM, 1927–89

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**ABSTRACT** Paralytic shellfish poisoning (PSP) was made a reportable condition in California in 1927. Since then, 510 cases, including 32 deaths, have been reported to California health authorities. Expansion of California's commercial shellfishing industry and growing numbers of people involved in sport shellfishing activities in recent years have heightened concerns about PSP and prompted the authors to review the State's PSP Prevention Program.

The California PSP Prevention Program is the oldest such program in the United States and is currently comprised of: (1) a mandatory reporting requirement for PSP, (2) an annual quarantine on sport-harvested mussels, (3) a coastal shellfish monitoring program, (4) PSP testing requirements for commercial shellfish harvesters, and (5) a public information program. Overall, the program appears to have been successful in controlling the occurrence of PSP in California, but it is challenged by the seemingly changing pattern of dinoflagellate blooms along California's coast and a substantial migration to California of persons unfamiliar with PSP or accustomed to harvesting shellfish in areas where PSP is not a problem.

This report presents information on the seasonal occurrence, geographic distribution, and types of shellfish involved in PSP illnesses in California. In addition, data from the coastal shellfish monitoring program are used to describe the frequency, intensity, seasonal occurrence, geography, and dynamics of toxic dinoflagellate blooms along the California coast. Also noted are differences in toxin levels in mussels, oysters, and other types of shellfish exposed to the same bloom conditions. Toxic bloom characteristics and changes in commercial and sport shellfishing activities are discussed in relation to PSP risks and the State's PSP Prevention Program.

**KEY WORDS:** PSP, *Protogonyaulax*, *Gonyaulax*, oysters, *Crassostrea*, mussels, *Mytilus*, shellfish.

### INTRODUCTION

Paralytic shellfish poisoning (PSP) is an acute, sometimes fatal form of food poisoning that has been a public health concern in California for many decades. The syndrome is caused by eating mussels, clams, oysters, and other filter-feeding marine bivalve mollusks that have become poisonous from ingesting certain marine dinoflagellates that contain saxitoxin and related biotoxins. The dinoflagellate of concern along California's coast is *Protogonyaulax catenella* (Whedon and Kofoid 1936, Taylor 1979), formerly known as *Gonyaulax catenella*.

*P. catenella* normally exists in the marine environment as dormant cysts in bottom sediments or as motile cells comprising a minor part of the plankton community. Under certain conditions, however, the dormant cysts may germinate, and the resulting motile forms, or active cells already present in low concentration, undergo periods of rapid pop-

ulation growth (Dale and Yentsch 1978, Anderson 1984). During these periods of proliferation, or dinoflagellate "blooms," coastal shellfish may become toxic.

Because most marine dinoflagellates possess brown or reddish pigments, dense populations in bloom areas may cause the ocean to take on a similar color. From this appearance has developed the popular association of poisonous shellfish with so-called "red tides." However, many red tides are caused by nontoxic dinoflagellates, and in California water *P. catenella* may be abundant enough to cause toxicity problems in shellfish without causing noticeable discoloration of the water (Carlisle 1968, Price 1989).

Saxitoxin and the related compounds causing PSP are among the most potent poisons known (Shimizu 1979, Hall and Reichardt 1984). The oral LD<sub>50</sub> of saxitoxin for mice is about 263 µg/kg (WHO 1984). A fatal oral dose for humans is only a few milligrams. Like tetrodotoxin, saxitoxin blocks the sodium channels of nerve membranes, and thereby inhibits neuronal transmission (Evans 1975, Schantz 1986; WHO 1984).

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Symptoms of PSP usually begin within 15 to 90 minutes after ingesting toxic mollusks and most often consist of paresthesia and numbness of the tongue, lips, and fingertips. If the dose is sufficient, this may be followed by muscular incoordination, a "floating" sensation, diplopia, dysarthria, and, in advanced cases, ascending paralysis. Death may result from diaphragmatic paralysis and respiratory failure within 2 to 12 hours (CDHS 1983, Halstead and Schantz 1984).

The clinical manifestations of PSP and the severity of symptoms depend upon the concentration of the toxin in the shellfish and the amount of shellfish consumed. There also appears to be substantial individual variation in susceptibility to the toxin. However, available data do not support the belief (McFarren et al. 1960) that persons who commonly eat shellfish in affected areas may develop resistance to the toxin (Halstead and Schantz 1984).

According to Tennant et al. (1955), severe symptoms have occurred following ingestion of as little as 124  $\mu\text{g}$  of toxin, and death has resulted from the ingestion of about 456  $\mu\text{g}$ . Bond and Medcof (1957) cite a case of a two-year-old child suffering serious poisoning after ingestion of clams containing only 96  $\mu\text{g}$ . In the same incident, two adults had mild symptoms after consuming an estimated 340 and 344  $\mu\text{g}$  each, and a severe poisoning resulted from ingestion of about 880  $\mu\text{g}$ . Evans (1975) states that symptoms usually are not felt by adults unless more than 360  $\mu\text{g}$  (2,000 mouse units) are ingested, while serious illness is likely following ingestion of more than 900  $\mu\text{g}$  (5,000 mouse units). Schantz et al. (1975) propose that severe illness, and even death, may occur after ingestion of 500 to 1,000  $\mu\text{g}$ . At the more conservative end, Dale and Yentsch (1978) suggest that moderate symptoms should appear upon the ingestion of 1,000  $\mu\text{g}$  of toxin, with the lethal range beginning at about 10,000  $\mu\text{g}$ .

Although the literature offers a range of opinions regarding the clinical implications of ingesting various levels of PSP toxin, and while there seem to be wide differences in human susceptibility to the toxin, it is reasonable to extrapolate that ingestion of 200 to 500  $\mu\text{g}$  is likely to cause at least mild symptoms; ingestion of 500 to 2,000  $\mu\text{g}$  is likely to cause moderate to severe symptoms; and consumption of more than 2,000  $\mu\text{g}$  is likely to produce serious, and possibly lethal, consequences.

CDHS recognizes that dose-response studies tend to concentrate on average dosages required to produce symptoms of various severities, rather than on minimum dosages which may affect highly susceptible persons. As stated by Bond and Medcof (1957): "... such averages can hold only academic interest in the minds of those responsible for preserving public health. They must concern themselves not merely with protecting the average person, but also those who are more susceptible to the poison than average."

All testing for PSP in California is done by the Cali-

fornia Department of Health Services, with toxin levels being determined by the standard mouse bioassay method (AOAC 1984).

The detection level for PSP toxins, using the mouse bioassay, is about 40  $\mu\text{g}$  per 100 grams of shellfish meat. The U.S. Food and Drug Administration "alert level" for PSP toxin in commercial shellfish is set at 80  $\mu\text{g}$ . That is, shellfish having levels of toxin at or above 80  $\mu\text{g}/100\text{ g}$  are considered unsafe and not allowed for sale (Clem 1975, NSSP 1988). This alert level is purposefully conservative to allow time to implement regulatory actions (e.g., closure of commercial shellfishing beds or recall of product from the marketplace) before toxin levels increase to more dangerous concentrations.

### Historical Background

PSP has been recognized as a health risk to consumers of bivalve shellfish in the temperate zones of North America and Europe for many centuries. Reports dating back to the eighteenth century describe numerous human illnesses resembling PSP following consumption of shellfish in Northern Europe and Great Britain (Halstead 1965).

In North America, Indians of the Pacific Coast apparently were aware of the relationship between red tides and bioluminescence in ocean waters and toxicity in shellfish. They would not eat shellfish when these conditions appeared (Meyer et al. 1928). Illustrative of this, in 1928, Meyer et al. wrote:

"From time immemorial it has been the custom among coast tribes of Indians, particularly the Poma, to place sentries on watch for Kal ko-o (mussel poison). Luminescence of the waves, which appeared rarely and then only during very hot weather, caused shellfishing to be forbidden for two days; those eating shellfish caught at such times suffered sickness and death. . . . According to a report a band of Indians died about fifty years ago from eating mussels gathered on the Mendocino coast during the month of August."

The first seemingly documented case of PSP in North America occurred in 1793, when five members of Captain George Vancouver's crew became ill after eating mussels collected near Fitzhugh Sound in what is now British Columbia (Quayle 1969). One of the five died. Vancouver's account of this event appears to be the first detailed written description of the symptoms and progression of paralytic shellfish poisoning (Vancouver 1801).

In 1799, it is reported that 100 Aleut hunters accompanying the Russian Baranoff Expedition died after eating a meal of mussels collected near present-day Sitka, Alaska (Halstead 1965). This event is not well documented, and some have suggested that the descriptions given are not sufficiently detailed to exclude other possible causes (McFarren et al. 1960). If this outbreak actually was PSP, it represents, by far, the greatest number of fatalities ever known to have resulted from a single PSP outbreak. Although there are no official reports of PSP incidents in Cali-

fornia during the early years of European settlement. Meyer et al. (1928) and Sommer and Meyer (1937) state that PSP cases were not uncommon during the latter half of the nineteenth century in California. Sommer and Meyer (1937) wrote:

"In order to visualize the significance of shellfish poisoning as a problem of health on the Pacific Coast, it is well to remember the following facts: During and following the investigation of the outbreak that occurred in 1927, numerous communications from physicians and other persons brought out the fact that mussel poisoning along the northern coast of California is a rather common phenomenon. Besides those recorded previously, numerous single and group intoxications are recalled by the inhabitants. Mass intoxications in Indians and single ones in white settlers which occurred about fifty years ago are also well remembered."

The first PSP outbreak recorded in California, and for the United States outside Alaska, involved 12 persons who ate mussels from Timber Cove, Sonoma County, in 1903 (Meyer et al. 1928). Five of these persons died. Subsequently, 4 cases were reported from Santa Cruz County in 1915, and 12 cases, including 2 deaths, in 1917. An additional case in San Diego County was reported in 1918.

It was not until 1927, however, that health officials in California fully recognized the serious potential of PSP. In that year, a major outbreak occurred along the San Francisco coast, extending from Sonoma to Monterey Counties. In total, 102 cases were reported, including 6 deaths (Meyer et al. 1928).<sup>†</sup> As a result of this outbreak, PSP was made a mandatorily reportable condition in California.

Since the 1927 California epidemic, interest in PSP in the United States and worldwide, has waxed and waned, with periodic reports being made of PSP incidents (Meyer et al. 1928, McFarren et al. 1960, Halstead 1965, and Rippey and Verber 1986). On reviewing the worldwide data, it is clear that the California coast is a region of especially high risk for PSP. It is an area which ranks high in the number of reported PSP cases and deaths (Table 1). Despite this, and the fact that California's PSP Prevention Program is the oldest such program in the United States, California's experience in preventing this public health problem has not been previously reported.

#### CALIFORNIA PSP PREVENTION PROGRAM

Since its inception over sixty years ago, the California Department of Health Services (CDHS) PSP Prevention Program has incrementally evolved into its current five-pronged approach, consisting of a mandatory reporting requirement for PSP cases, an annual mussel quarantine, a

<sup>†</sup>Meyer et al. 1928, and Sommer & Meyer, 1937, both give 102 as the total number of cases in the 1927 outbreak. Some time later, a single additional case was attributed to this year, giving a total of 103. As noted by Meyer et al. 1928, the total of 102 "is only approximate" and "it is not unlikely that only the severe and moderately severe forms of illness have been recognized and tabulated."

TABLE 1.

Number of reported incidents of PSP in North America during the period 1900 through 1989.<sup>a</sup>

Area	PSP Illnesses (Deaths)
Alaska	174 (7)
British Columbia	103 (5)
Washington	36 (3)
Oregon	>22 (1)
California	539 (39)
Mexico <sup>1</sup>	>18 (3)
Guatemala <sup>2</sup>	>175 (26)
Rhode Island	1
Massachusetts	73
New Hampshire	6
Maine	>98
Nova Scotia and New Brunswick	107 (2)
Quebec	46 (4)
New Foundland	1

<sup>1</sup> A single outbreak near Mazatlan, Sinaloa, in 1979. The causal dinoflagellate was identified as *Gymnodinium catenatum* Graham.

<sup>2</sup> A single outbreak near Champerico, Retalhuleu, on the Pacific coast of Guatemala in 1987. The causal dinoflagellate was identified as *Pyrodinium bahamense* var. *compressa*.

<sup>a</sup> From Rippey and Verber, 1986, plus recent records. Illnesses and deaths from neurotoxic shellfish poisoning (NSP) and amnesic shellfish poisoning (ASP) are not included.

coastal shellfish monitoring program, testing requirement for commercial shellfish harvesters, and a public information program (CDHS 1989). Closure of commercial shellfishing operations, special quarantines of defined areas, and public warnings against consumption of sport-harvested bivalves are based on the findings of the coastal monitoring and commercial testing programs. These elements are described below.

#### 1. Mandatory Reporting of PSP Cases

As already noted, PSP was made a mandatorily reportable condition in California in 1927. Since then, 510 cases, including 32 deaths, have been reported to CDHS. A summary of these cases is given in Table 2.

The number of cases associated with various kinds of shellfish is shown in Table 3.

#### 2. Annual Mussel Quarantine

CDHS initiated its annual six-month (May 1 to October 31) mussel quarantine in 1942 (CDPH 1942). This action followed previous more limited quarantines and public warnings about the eating of mussels and clams during the summer months. These earlier quarantines, dating back to 1927, were based on shellfish testing results and occurrences of PSP illnesses, and they covered different periods each year. The known danger period was gradually expanded from July and August to its current May through October period as elevated shellfish toxin levels and human PSP cases were reported earlier and later in the year.

TABLE 2.  
Reported human cases of paralytic shellfish poisoning in California, 1927-89.

Year	Cases	Deaths	Counties Involved <sup>1</sup>	Type of Shellfish
1927	103	6	Sonoma, Marin, San Mateo	Mussels
1929	60	4	Sonoma, Marin, San Mateo	Mussels (54 cases, 1 death) Clams (6 cases, 3 deaths)
1930	2	0	Sonoma	Mussels
1931	2	0	Unknown	Mussels
1932	44	2	Mendocino, Sonoma, Marin, San Mateo, Monterey	Mussels (41 cases, 1 death) Clams (2 cases, 0 deaths) Unknown (1 case, 1 death)
1933	12	0	Del Norte, San Mateo	Mussels
1936	3	2	Ventura	Mussels
1937	27	0	Sonoma, Santa Cruz, Monterey	Mussels
1938	3	0	Ventura	Mussels
1939	76	8	Santa Cruz, Monterey	Mussels (64 cases, 6 deaths) Clams (12 cases, 2 deaths)
1943	20	4	Del Norte, Humboldt	Mussels
1944	12	2	San Mateo, Santa Cruz	Mussels
1946	3	1	San Mateo	Mussels
1947	1	0	San Mateo	Clams
1948	3	1	San Mateo	Mussels
1954	5	0	Sonoma, Marin	Mussels
1962	4	0	Marin	Oysters
1969	15	0	Humboldt, Mendocino	Mussels
1971	15	0	Humboldt, San Francisco, San Mateo	Mussels
1980	98	2	Sonoma, Marin	Mussels (36 cases, 1 death) Oysters (61 cases, 0 deaths) Scallops (1 case, 1 death)
1989	2	0	Mendocino	Mussels
TOTAL	510	32		

<sup>1</sup> Probable source of the shellfish causing the PSP illness.

The early quarantines applied to designated sections of the coast, initially from Monterey to Del Norte Counties. In 1936, the southern boundary was extended to include Ventura County, following the occurrence of three cases, including two deaths, from mussels collected in that county in May of that year. The following year, Los Angeles County also was included. The early quarantines specifically excluded San Francisco Bay. It was not until 1942 that San Francisco Bay and the entire coast of California, from Oregon to Mexico, were included in the quarantine. Also in that year, while the quarantine remained limited to

mussels, it included an advisory about discarding the dark meat, or viscera, of clams before eating them.

The annual quarantine order is a preventive measure and is routinely issued each year. Since PSP toxin levels can increase rapidly and unpredictably, especially in mussels, and since the approximately 1,000-mile length of California's coastline makes it virtually impossible to adequately sample for PSP activity with available resources, CDHS believes the order is well justified. The annual quarantine is restricted to mussels because of their common occurrence along the open coast where they are quickly exposed to oceanic blooms of toxic dinoflagellates, and because mussels tend to acquire the toxin more rapidly and to concentrate it at higher levels than other kinds of shellfish. Also, as noted in Table 3, mussels have been responsible for the majority of California PSP cases (83 percent) and deaths (78 percent).

The annual quarantine is supplemented by an ongoing coastal shellfish sampling program. Information on PSP levels obtained from this program is used to modify or expand the quarantine, as necessary—i.e., either the time period or the kinds of mollusks covered.

The quarantine applies only to sport-harvested mussels. Mussels and all other bivalves grown and harvested by li-

TABLE 3.  
California's PSP cases according to type of shellfish, 1927-89.

	Cases	Deaths
Mussels	422	25
Clams	21	5
Oysters	65	0
Scallops	1	1
Unknown	1	1
TOTAL	510	32

censed commercial operators in California are subject to a separate PSP testing program, as described below. Commercial shellfish entering California from other states are subject to similar controls.

### 3. Coastal Shellfish Monitoring

Although blooms of *P. catenella* in California's coastal waters generally occur during the warmer months of the year, there is insufficient understanding of the environmental and biological factors affecting *P. catenella* populations to adequately predict bloom occurrences (Yentsch 1984). Available information only confirms that toxic blooms along the California coast are very irregular, both with respect to place and time. Because their appearance cannot now be predicted, nor their presence detected by other means, California, like most other states with PSP problems, relies on a coastal shellfish monitoring program to detect bloom developments (Nishitani and Chew 1988, Chiang 1988, Shumway et al. 1988).

Figures 1 and 2 show the locations of the 15 coastal counties of California, the principal commercial shellfish harvesting areas, and selected coastal sampling sites referred to in this report.

During the period 1927-36, a shellfish sampling program using the newly developed mouse bioassay was conducted by the G. W. Hooper Foundation of the University of California, San Francisco, as part of the pioneering studies of PSP. The results of this sampling effort were described by Sommer and Meyer (1937).

Records of shellfish testing for PSP during the 1940s and 1950s are incomplete and difficult to interpret. It appears that during these two decades little or no PSP testing was performed in a number of years. It was not until 1962 that the coastal monitoring program became an ongoing annual activity. Similarly, it was not until 1962 that PSP levels were expressed in micrograms ( $\mu\text{g}$ ) of toxin per 100 grams of shellfish tissue, rather than the earlier "liver units" and "mouse units." For these reasons, this report

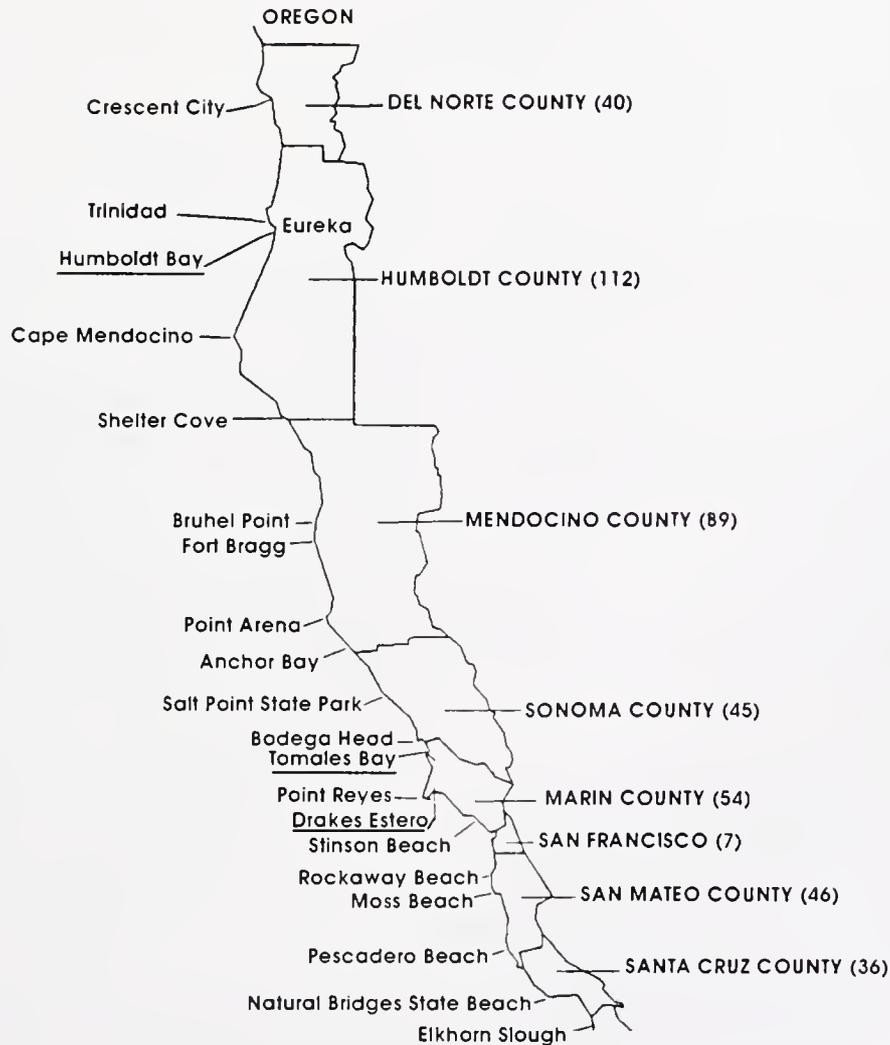


Figure 1. Central and Northern California coastal counties from Del Norte to Santa Cruz, with coastal mileages (in parentheses), commercial shellfish growing areas (underlined), and selected geographic locations and sampling sites.

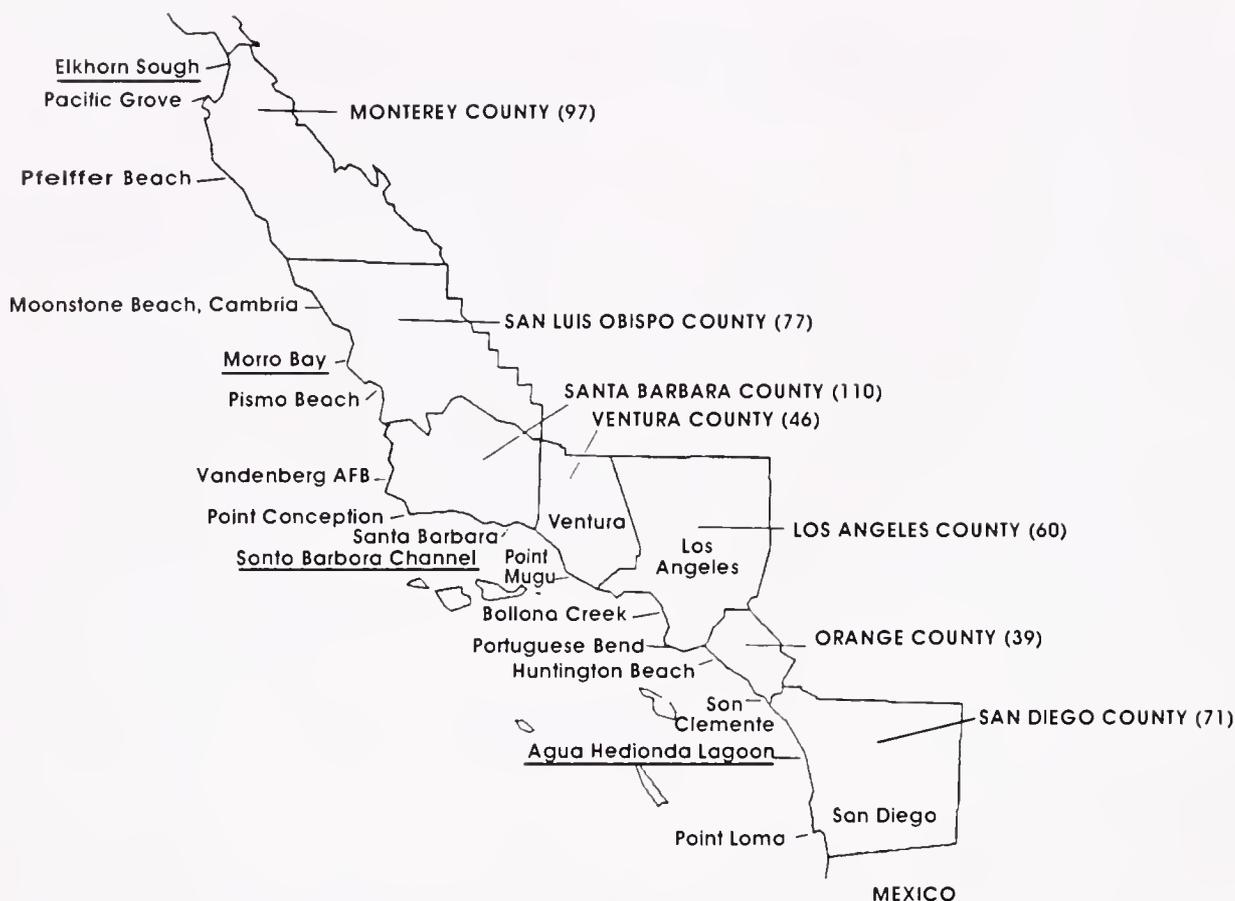


Figure 2. Central and Southern California coastal counties from Monterey to San Diego, with coastal mileages (in parentheses), commercial shellfish growing areas (underlined), and selected geographic locations and sampling sites.

summarizes and focuses primarily on the findings of the shellfish sampling program that began in 1962.

The monitoring program began as an informal agreement between the CDHS and several coastal county health departments. The latter would collect and submit shellfish samples to CDHS for PSP testing; CDHS would then analyze the samples and report the results back to the counties. The coastal counties did not all become a part of the monitoring effort at the same time. The program first involved the northern counties, and it later grew to include southern counties.

As the CDHS PSP Prevention Program evolved, a sampling protocol was formulated which suggested that each county select two sampling locations, and that a sample from each site be submitted at biweekly intervals during the quarantine period and at monthly intervals during the non-quarantine period. The guideline, if followed, would have provided for relatively regular and uniform geographic coverage of the coast. However, for various reasons, some coastal county health departments have not been able to participate in the sampling effort as fully as initially anticipated. As a result, the number of samples submitted by these local agencies varies widely, a fact which contributes

to the uneven sampling patterns which have characterized the monitoring program, especially in its early years. The total number of samples submitted for PSP analyses each year since 1962 is shown in Figure 3.

Monitoring by the coastal counties is augmented in some areas by samples collected by CDHS, the California Department of Fish and Game (CDFG), the University of California, Vandenberg Air Force Base, and various other program participants. In addition, samples are submitted by commercial shellfish harvesters as part of their permit requirements.

The number of samples submitted by various program participants during each of the past five years is shown in Table 4. The average number of samples submitted annually from each of the 15 coastal counties during the same time period is shown in Figure 4. The wide range in numbers of samples submitted from each county reflects, in part, the length of the coastline, the involvement of local health agencies, the presence or absence of commercial shellfishing operations, and the size and nature of shellfish resource. Also, some areas, for example parts of Monterey, Mendocino, and Humboldt counties, are relatively remote and inaccessible for routine sampling activities.

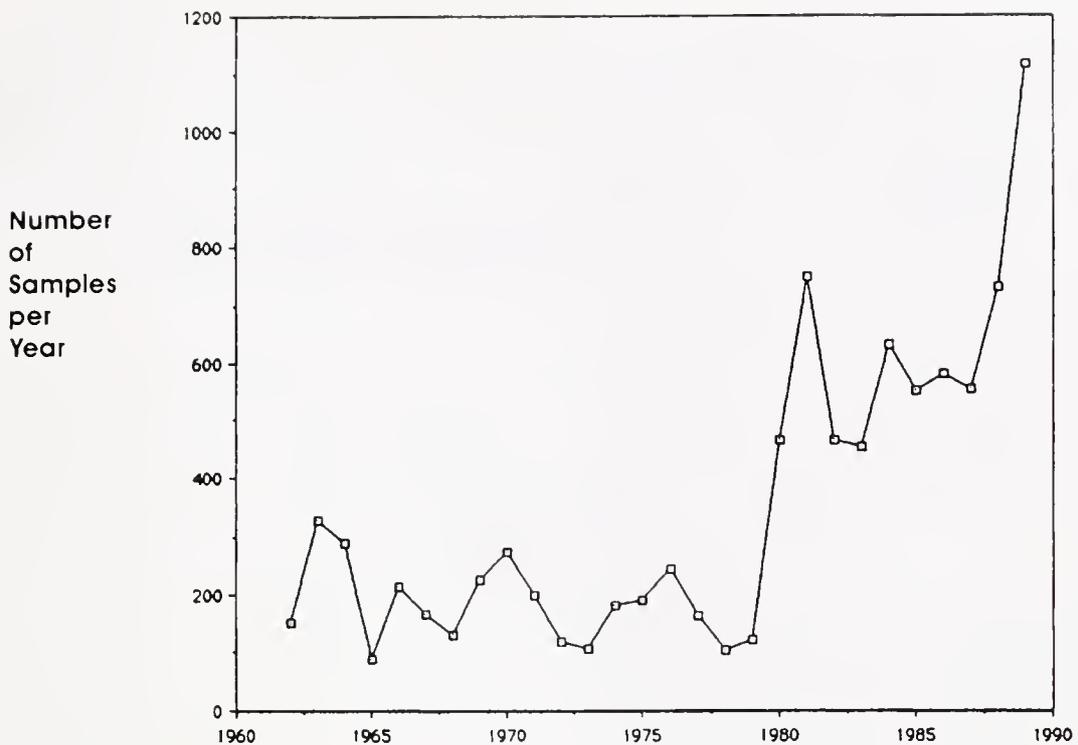


Figure 3. Total number of shellfish samples submitted annually to the California Department of Health Services for paralytic shellfish poison (PSP) analysis during the period 1962-1989

Because toxic dinoflagellate blooms in California begin in ocean waters and secondarily are carried into bays and estuaries through currents and tidal action, shellfish toxin levels in bays and estuaries generally are lower than those in adjacent open-coastal locations. It follows, therefore, that samples from the open coast have greater value in an early warning system for toxic bloom detection than do those from protected waters. This is why sampling has been targeted to open coastal locations.

In 1988, the CDHS adopted a sampling technique in which mussels or other shellfish are placed in retrievable nylon mesh bags hung into ocean waters from piers or other suitable structures. These so-called sentinel stations offer safety advantages to the sampler in that the bags can be retrieved at any time independent of the need for low tides or suitable weather and surf conditions. Use of this procedure is limited because most of the open coast lacks piers or other structures overhanging the water from which bags can be hung.

Primary sentinel stations are located at Trinidad, Humboldt County; the U.S. Coast Guard Station in Humboldt Bay; Lawson's Landing in Tomales Bay; and at the Old Coast Guard Station near Chimney Rock, Drakes Bay.

#### 4. Shellfish Sampling from Commercial Harvesting Areas

The coastal shellfish monitoring program was expanded in 1981 when CDHS required that commercial shellfish growers submit samples from their shellfishing beds at

weekly intervals, year-round, during all harvesting periods. This requirement was imposed as a consequence of a PSP outbreak in 1980 during which 61 people became ill from eating commercially grown oysters. Even before 1981,

TABLE 4.

Number of PSP samples submitted annually during the period 1985-1989 by commercial shellfish growers, government agencies, research institutions, and other program participants.

	1985	1986	1987	1988	1989
Commercial Shellfish					
Growers	249	295	298	364	550
Local Health Departments	183	176	170	191	171
Department of Health Services	—	8	6	82	230
Department of Fish and Game	11	8	8	11	50
U.C. Bodega Marine Laboratory	22	23	15	27	29
Vandenberg Air Force Base	25	24	25	30	28
Golden Gate National Recreational Area	—	—	4	7	18
Other Program Participants <sup>1</sup>	63	49	29	20	46
Total	553	583	555	732	1,122

<sup>1</sup> Other program participants include Lawson's Landing Resort, Tomales Bay, Marin County; Point Reyes Bird Observatory, Marin County; Fitzgerald Marine Reserve, San Mateo County; Orange County Marine Institute; California Department of Parks and Recreation; U.S. Naval Air Station, Point Mugu, Ventura County; and Neushul Mariculture, Santa Barbara County.

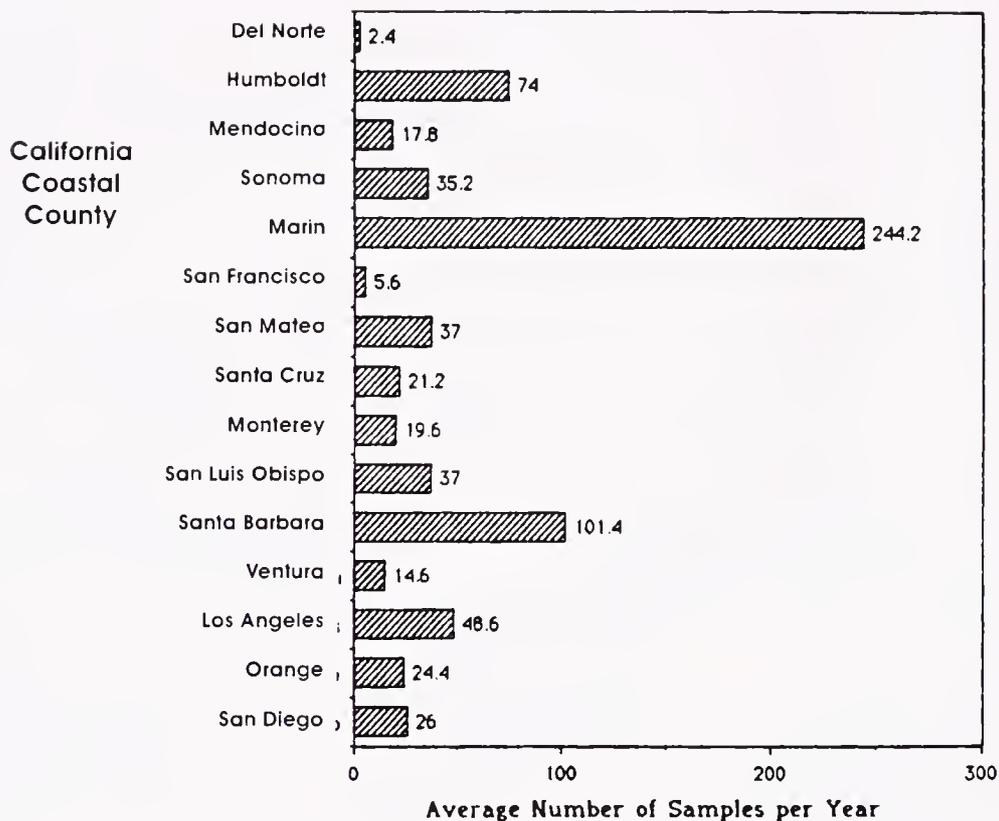


Figure 4. Average number of samples submitted for paralytic shellfish poison (PSP) analysis from each California coastal county during the period 1985–1989

however, samples commonly were sent to CDHS for testing by shellfish growers, particularly from Humboldt Bay, Tomales Bay, and Drakes Estero. Although not mandatory, sampling was done voluntarily to ensure product safety. In recent years, about one-half of all samples received for PSP testing have been submitted by commercial shellfishing companies (Table 4).

Year-round testing of commercially grown shellfish not only helps to protect the consumer, but is essential for the development and economic success of the State's expanding shellfishing industry. PSP outbreaks, such as occurred in New England in 1972 and California in 1980, can have protean untoward impacts on shellfish markets, as well as on the general fisheries industry (Jensen 1975, Conte 1984). An overview of the economic effects of PSP and costs of PSP prevention programs on the North American shellfishing industry is given by Lutz and Incze (1979). The cost of the California PSP Prevention Program is borne, in part by the State's general fund as a public health protection program, and, in part, by license fees paid to the Department of Fish and Game.

##### 5. Public Education

The public education effort is directed primarily toward sport shellfish harvesters and is closely linked to the annual

mussel quarantine. Opening of the annual quarantine period is widely announced in the media by means of CDHS press advisories. These news releases contain a statement of the quarantine, the reasons for it, and background information on PSP. Extensions of the quarantine beyond the normal closing date, inclusion of shellfish other than mussels, and all other emergency actions are also announced by press advisories and public notices.

CDHS keeps all local health agencies, the CDFG, the California Department of Parks and Recreation, and other relevant entities informed about coastal PSP activity and the status of all quarantines so they can assist in enforcement and public education activities.

As part of the public information effort, quarantine warning signs are prepared and posted in shellfishing areas by local health departments. Since 1980, in addition to being printed in English and Spanish, signs also have been printed in Vietnamese, Cambodian, Laotian, Hlocano, and Tagalog. Special effort is made to reach recent immigrants from the Philippines and Southeast Asia, since these groups have accounted for a disproportionate number of PSP cases in California in recent outbreaks. For example, in 1980, 13 of 36 cases due to the consumption of mussels were Filipinos (Sharpe 1981). Also, of the 15 reported cases in each of the outbreaks of 1969 and 1971, 12 in each were Fili-

pinos. The high incidence among Filipinos, and to a lesser extent among immigrants from Southeast Asian countries, may be explained by a cultural dietary penchant for mussels and other shellfish and unfamiliarity with PSP, which rarely occurs in that part of the world.

In addition to CDHS public education efforts, the University of California Sea Grant Extension Program publishes an informational bulletin on PSP for the general public (Price 1989).

#### FINDINGS OF THE COASTAL MONITORING PROGRAM

In spite of the high level of PSP activity along the California coast, there has been almost no academic research on this problem in the State since the early studies by Meyer, Sommer, and their co-workers in the late 1920s and 1930s. Instead, recent advances in the understanding of this phenomenon in California have come primarily from the CDHS coastal shellfish monitoring program and other health protection activities.

Although, as already noted, the monitoring effort has been somewhat sporadic since being initiated in 1962, the sampling data from recent years are yielding important new information on the frequency, intensity, seasonal occurrence, geographic distribution, dimensions, and other related aspects of toxic dinoflagellate blooms and toxification of shellfish along the California coast. Eventually, these data may permit correlation of toxic blooms with climatic and hydrographic conditions which, in turn, might lead to prediction capabilities. Until then, however, the monitoring data are providing useful clues concerning the ecology and dynamics of toxic blooms—information essential in developing optimal sampling strategies, predicting the course of bloom expansion and decline, and, in general, providing a more effective public health protection program. Some of these findings are described below.

#### Frequency of PSP Blooms in California

A "bloom" may be defined as a discrete proliferation of toxic dinoflagellates which causes PSP toxin levels in exposed shellfish to rise above some detectable level. Because a bloom is detected indirectly (i.e., by measuring toxin in shellfish), our knowledge of bloom frequencies is limited by the number and distribution of sampling sites along the coast and the frequency of sampling. Although limited, data available since 1962 permit an estimate of this aspect of bloom occurrences.

A review of sampling data from California reveals that for most years samples measuring 80  $\mu\text{g}/100\text{ g}$  (the federal FDA alert level) or more are very common, occurring too frequently to provide meaningful information on toxic bloom frequencies. For that reason, CDHS has operationally defined a bloom as occurring at levels of 160  $\mu\text{g}/100\text{ g}$  or more. Also, a discrete bloom is defined as one which is separate from other similar events, both in location and time of occurrence. In some years, because of spatial and

temporal gaps in sampling data, it is not possible to determine if elevated toxin levels in coastal shellfish are the result of a single large bloom or of smaller discrete blooms. Table 5 shows the number of discrete toxic blooms, as defined here, detected along the California coast from 1962 through 1989, along with the location of the bloom, the months of occurrence, and the highest toxin level obtained for each county involved in the bloom.

It is clear from the data in Table 5 that toxic dinoflagellate blooms commonly occur off the California coast, having recorded 34 distinct blooms between January 1962 and December 1989, inclusive. In only 6 of 28 years was no bloom identified. In 10 years, 2 or more toxic blooms were detected. However, because of limited sampling, especially in the years prior to 1980, it is likely that many blooms went undetected.

#### Intensity of PSP Blooms in California

Sampling data (Table 5) have shown that toxic dinoflagellate blooms not only are relatively common along the California coast, but that shellfish toxicity also frequently reaches levels that could cause serious health problems if it were consumed. Table 5 shows that of 34 identified blooms, 31 (91 percent) yielded shellfish with toxin levels of 200  $\mu\text{g}/100\text{ g}$  or higher; 17 (50 percent) yielded samples of 500  $\mu\text{g}/100\text{ g}$  or higher; and 8 (24 percent) yielded specimens having levels of 2,000  $\mu\text{g}/100\text{ g}$  or higher.

Bloom intensities also may be described by the highest shellfish toxin levels encountered. As shown in Table 6, the federal alert level of 80  $\mu\text{g}/100\text{ g}$  has been exceeded in one or more locations in 26 of the past 28 years (93 percent). Levels have equaled or exceeded 200  $\mu\text{g}/100\text{ g}$  in 21 of the past 28 years (75 percent), 500  $\mu\text{g}/100\text{ g}$  in 14 years (50 percent), and 2,000  $\mu\text{g}/100\text{ g}$  in 8 years (29 percent). It is also of interest that levels have exceeded 1,000  $\mu\text{g}/100\text{ g}$  in 6 of the past 10 years. The reason for detecting higher toxin levels during recent years is unclear. It is possible that this is an artifact of more intense sampling, rather than of any real increase in high-level toxic blooms.

#### Seasonal Occurrence of PSP Blooms in California

As previously noted, the annual mussel quarantine period evolved during the 1930s to encompass those months having the highest PSP risks. The period chosen in 1942, May 1 through October 31, has included over 99 percent of all reported PSP cases in California since 1927. The only exceptions were two cases in March 1931, and one case (fatal) in April 1932 (Sharpe 1981). The two largest PSP outbreaks in California, in 1927 and 1980, began during the month of July. As can be seen from Table 7, the overwhelming majority of PSP cases from other years (94 percent) have occurred during the 4-month period June through September, with July being the peak single month (58 percent) (Sommer and Meyer 1937; Sharpe 1981). June, July, and August also lead in the number of PSP

TABLE 5.  
Toxic dinoflagellate blooms along the California coast, 1962-89.\*

Year	Counties Involved	Highest Toxin Level <sup>1</sup>	Location	Date
1962	Sonoma	1,200	Bodega Bay	08/24/62
	Marin	1,920	Drakes Estero	07/28/62
1962	Mendocino	428	Mendocino Coast	10/25/62
1963	Marin	400	Drakes Estero	07/18/63
1965	Marin	340	Drakes Estero	08/30/65
1966	Marin	360	Drakes Estero	01/28/66
1966	Mendocino	1,600	Mendocino Coast	08/25/66
	Marin	250	Drakes Estero	07/26/66
1967	Mendocino	240	6m. N. Ft. Bragg	08/31/67
1968	Sonoma	170	Horseshoe Cove	07/10/68
1968	Sonoma	200	Horseshoe Cave	09/06/68
1969	Humboldt	2,300	Shelter Cove	09/23/69
	Mendocino	1,900	Fort Bragg	10/01/69
	Sonoma	170	Salt Point	10/01/69
1970	Sonoma	240	Ocean Cove	02/18/70
1970	Sonoma	210	Mussel Point	06/24/70
	Marin	280	Stinson Beach	06/24/70
	San Francisco	760	Lands End	07/21/70
	San Mateo	2,800	Graywhale Cove	07/20/70
1971	Humboldt	850	Trinidad	09/09/71
	Sonoma	510	Bodega Head	08/11/71
	Marin	290	Drakes Estero	07/28/71
	San Francisco	3,200	Lands End	08/10/71
	San Mateo	6,000	Moss Beach	08/05/71
	Santa Cruz	340	Natural Bridges	08/10/71
1973	Humboldt	162	College Cove	07/18/73
1975	Mendocino	498	Mussel Point	09/10/75
1976	Sonoma	200	Sea Ranch	03/11/76
	Marin	240	Stinson Beach	03/12/76
1976	Sonoma	280	Bodega Head	07/30/76 and 08/10/76
1980	Ventura	309	Bass Rock	05/19/80
1980	Sonoma	14,000	Duncan's Landing	07/26/80
	Marin	16,000	Tomaes Bay	07/29/80
	San Francisco	500	Phelan Beach	07/31/80
1980	Mendocino	330	Mussel Rock	11/18/80
1981	Sonoma	2,200	Schoolhouse Beach	10/13/81
	Marin	870	Dillon Beach	10/09/81
1982	Mendocino	220	Bell Point	09/02/82
	Sonoma	640	Schoolhouse Beach	09/03/82
	Marin	690	Rodeo Cove	08/18/82
	San Mateo	470	Graywhale Cove	08/27/82
1983	San Francisco	210	Baker Beach	07/26/83
	San Mateo	240	Pillar Point	07/14/83
1984	Sonoma	190	Mussel Point	03/11/84
	Marin	4,000	Chimney Rock	03/08/84
	San Francisco	520	Yerba Buena Island	03/16/84
	San Mateo	670	Moss Beach	03/14/84
	Santa Cruz	170	Natural Bridges	03/14/84

TABLE 5.  
continued

Year	Counties Involved	Highest Toxin Level <sup>1</sup>	Location	Date
1984	Mendocino	1,520	Bruhel Point	08/29/84
	Sonoma	180	Bodega Head	08/28/84
	Marin	680	Kehoe Beach	08/27/74
1985	San Luis Obispo	340	Moonstone Beach	04/08/85
	Santa Barbara	4,100	Santa Barbara Channel	04/10/85
	Ventura	300	Mobil Pier	04/05/85
1986	Marin	300	Drakes Estero	04/09/86
1986	Marin	1,900	Drakes Estero	06/18/86
	Marin	1,900	Rodeo Cove	06/27/86
	San Francisco	400	China Beach	06/23/86
	San Mateo	390	Graywhale Cove	06/24/86
1987	Marin	180	Chimney Rock	05/19/87
1987	Sonoma	500	Mussel Point	08/11/87
	Marin	440	Drakes Estero	07/24/87
	San Mateo	270	Point San Pedro	07/28/87
1988	Marin	810	Chimney Rock	08/01/88
	San Mateo	330	Ano Nuevo State Beach	07/14/88
1989	Marin	1,800	Chimney Rock	01/10/89
1989	Humboldt	14,000	Humboldt Bay	09/18/89
	Mendocino	6,300	Anchor Bay	09/17/89
	Sonoma	5,500	Salt Point St. Park	09/24/89
	Marin	1,800	Tomales Bay	09/07/89
	Marin	1,800	Point Reyes N.S.	09/19/89
	San Mateo	800	Moss Beach	08/20/89
	Santa Cruz	930	Near San Mateo Co. Line	08/18/89
1989	San Luis Obispo	1,600	Cayucos	12/13/89

<sup>1</sup> In micrograms toxin per 100 g of shellfish meat. All samples are mussels except those from Drakes Estero, Marin County, in 1962, 1963, 1965, 1966 (2 samples), and 1971, which were oysters.

<sup>2</sup> From CDHS Shellfish Monitoring Program: no blooms detected in years not listed. Only samples of 160 ug/100 g or greater are included. Clam samples are excluded.

deaths (84 percent) (Table 7), and 31 of the deaths (97 percent) have occurred during the May–October quarantine period.

Although the mussel quarantine covers that part of the year during which the vast majority of PSP cases have occurred, the CDHS sampling program has revealed that toxic dinoflagellate blooms are not limited to only the warmer months of the year. Toxic blooms have caused numerous extensions of the quarantine period beyond October 31, and early-season quarantines prior to May 1 have not been unusual in recent years.

Table 8 shows the month of highest reported toxin levels for the 34 dinoflagellate blooms identified in Table 5. As expected, July and August lead in the number of bloom peaks (74 percent). Also, it is interesting that while a peak toxin level has been detected only one time during June, this month leads all, except July, in the number of reported PSP illnesses (Table 7).

The occurrence of toxic blooms during nonquarantine

months, although less frequent than those during the May–October period, underscores the need for a year-round coastal monitoring program and mandatory weekly sampling requirements for all commercial harvesters. Obviously, the occurrence of toxic blooms during nonquarantine months causes special public health concerns. Such occurrences must be detected and promptly acted upon.

Coastal shellfish toxicity in the three southernmost California counties (i.e., Los Angeles, Orange, and San Diego) has not exceeded the 160 µg/100 g level since monitoring began. However, if one tabulates minor elevations in toxicity (80 µg and above) from this part of the State, no seasonal patterns emerge. At least as many such events occurred during the nonquarantine period as during the quarantine period (Table 9). It seems important, therefore, that these counties maintain a uniform sampling effort throughout the year. In this area, it appears as though a toxic bloom could occur during any season of the year.

In the area immediately north of Los Angeles County,

TABLE 6.

Highest PSP toxin levels detected in the California shellfish monitoring program by year, 1962-89.

Year	Month	Highest Level <sup>1</sup>	County
1962	July	1,920 <sup>a</sup>	Marin
1963	July	400 <sup>a</sup>	Marin
1964	July	93 <sup>b</sup>	Marin
1965	August	340 <sup>a</sup>	Marin
1966	August	1,600	Mendocino
1967	August	240	Mendocino
1968	April	270 <sup>b</sup>	Sonoma
1969	September	2,300	Humboldt
1970	July	2,800	San Mateo
1971	August	6,000	San Mateo
1972	March	131	Los Angeles
1973	July	162	Humboldt
1974	May	92	Orange
1975	September	498	Mendocino
1976	July, August	280	Sonoma
1977	December	66	Ventura
1978	May	92	Santa Barbara
1979	September	68	San Luis Obispo
1980	July	16,000	Marin
1981	October	2,200	Sonoma
1982	August	690	Marin
1983	July	240	San Mateo
1984	March	4,000	Marin
1985	April	4,100	Santa Barbara
1986	June	1,900	Marin
1987	August	500	Sonoma
1988	August	810	Marin
1989	September	14,000 <sup>c</sup>	Humboldt
1989	September	6,300 <sup>d</sup>	Mendocino

<sup>1</sup> In micrograms of toxin per 100 grams of shellfish meat. Samples are mussels unless otherwise indicated.

<sup>a</sup> Oysters.

<sup>b</sup> Clams.

<sup>c</sup> Sentinel sea mussels.

<sup>d</sup> Wild sea mussels.

toxic blooms appear to be early season events. For example, the two reported blooms from Ventura County in 1980 and 1985 occurred during April and May (Table 5). Similarly, the intense and, to date, only toxic bloom recorded in Santa Barbara County occurred during March and April 1985 (Table 5). Also of note is the unusual bloom in San Luis Obispo County which occurred during December 1989 (Table 5).

#### Geographic Distribution of Toxic Dinoflagellates Along the California Coast

The California coast extends in straight-line measurements about 930 miles (1,497 kilometers) from Oregon to Mexico, a distance comparable to that from northern Maine to Virginia, although the actual coastline, including all bays and inlets, measures approximately 1,100 miles. Given this length, it is not surprising that toxic bloom ac-

TABLE 7.

Incidence of PSP illnesses by month, California, 1927-89.

Month	Cases		Deaths	
	Number	(%)	Number	(%)
January	0	(0.0)	0	(0.0)
February	0	(0.0)	0	(0.0)
March	2	(0.4)	0	(0.0)
April	1	(0.2)	1	(3.1)
May	7	(1.4)	2	(6.3)
June	82	(16.1)	8	(25.0)
July	293	(57.7)	10	(31.3)
August	67	(13.2)	9	(28.1)
September	36	(7.1)	0	(0.0)
October	20	(3.9)	2	(6.3)
November	0	(0.0)	0	(0.0)
December	0	(0.0)	0	(0.0)
TOTAL	508 <sup>a</sup>		32	

<sup>a</sup> Month of occurrence of two PSP illnesses in 1937, Santa Cruz County, is not available.

tivity shows considerable geographic differences in both frequency of occurrence and intensity.

In general, Northern California experiences significantly more PSP activity than does Southern California. Toxic bloom activity, however, does not increase linearly as one moves north, but rather tends to be concentrated in the north-central part of the State, especially in the area immediately north and south of San Francisco Bay. As shown in Table 10, Marin County has been the most frequent center of bloom activity, followed by Sonoma and Mendocino Counties. The five counties having the most frequent occurrence of toxic blooms are Mendocino, Sonoma, Marin, San Francisco, and San Mateo (Table 10). Collectively,

TABLE 8.

Month of highest reported PSP toxin level in coastal shellfish during each of 34 dinoflagellate blooms in California, 1962-89.

Month	Number of Bloom Peaks <sup>1</sup>
January	2
February	1
March	2
April	2
May	2
June	1
July	8
August	9
September	4
October	2
November	1
December	1
TOTAL	35

<sup>1</sup> A 1976 bloom had equal peak levels in both July and August.

TABLE 9.

Mussel samples with PSP toxin levels of 80 or more µg/100 g of shellfish meat from southern California, 1970-88.

Year	Date	Location	County	Toxin Level <sup>1</sup>
1970	May 28	Venice	Los Angeles	100
1971	June 7	Venice	Los Angeles	91
1972	March 9	Portuguese Bend	Los Angeles	131
1974	May 9	Corona Del Mar	Orange	92
1976	January 15	Huntington Beach	Orange	95
1976	January 15	Newport Beach	Orange	88
1980	April 14	San Clemente	Orange	111
1983	September 12	Portuguese Bend	Los Angeles	110
1984	May 17	Huntington Beach	Orange	100
1984	May 17	San Clemente	Orange	110
1985	January 6	Marina Del Rey	Los Angeles	125
1985	January 17	Portuguese Bend	Los Angeles	94
1985	March 22	La Jolla	San Diego	150
1985	April 2	Huntington Beach	Orange	91
1986	March 24	Portuguese Bend	Los Angeles	86
1987	April 7	Portuguese Bend	Los Angeles	96
1988	March 14	Portuguese Bend	Los Angeles	83

<sup>1</sup> In micrograms toxin per 100 g of shellfish meat.

these 5 counties have been involved in 83 percent of the 34 blooms recorded since 1962.

Table 11 shows the highest levels of PSP toxin reported from each of the 15 coastal counties during the period

TABLE 10.

Counties affected in 34 discrete toxic blooms, 1962-89.

County	Number of Occurrences	
	Bloom Center <sup>1</sup>	Bloom Involvement <sup>2</sup>
Del Norte	0	0
Humboldt	3	4
Mendocino	6	9
Sonoma	6	16
Marin	13	20
San Francisco	0	6
San Mateo	3	8
Santa Cruz	0	3
Monterey	0	0
San Luis Obispo	1	2
Santa Barbara	1	1
Ventura	1	2
Los Angeles	0	0
Orange	0	0
San Diego	0	0
Total	34	

<sup>1</sup> Number of times each county has had the highest reported level of PSP toxin in any given toxic bloom.

<sup>2</sup> Number of times each county has been included in a discrete bloom, as defined in text. Counties without sampling data during bloom periods are not included, and, thus, this may understate their actual occurrence.

1962-89, along with the number of years in which each county has been a part of the monitoring effort. As already noted, the monitoring program first involved the northern counties, and later those to the south. Del Norte County is an exception in that very few samples have been submitted from that area—a fact which explains the low PSP level in Table 11. The maximum levels reported reflect the higher overall PSP activity in Northern California.

Plots of the highest toxin levels encountered during each year of the monitoring program for each county permits a comparison of general PSP activity in discrete coastal areas. Such plots also show that toxic blooms sometimes occur in areas with little or no prior history of such occurrences. For example, as shown in Figure 5, the 1985 bloom event in Santa Barbara followed 15 years of monitoring (1970-1984) during which period the highest recorded level of PSP toxin was only 97 µg/100 g.

Similarly, the unusual bloom in December 1989, in San Luis Obispo County, occurred in an area of low PSP activity, as shown in Figure 6. Marin and Sonoma counties, by contrast, have more active histories of toxic blooms (Figures 7 and 8).

These records, although showing only the single highest toxin level per year, allow one to visualize the unpredictable nature of blooms in California. In addition, over a period of years, these types of data might reveal cycles of toxic bloom activity and long-term trends in the frequency and intensity of blooms along the California coast. Also,

TABLE 11.

Highest PSP toxin level reported by county, 1962-89.

County	Number of		Year
	Years Monitored <sup>1</sup>	PSP Level <sup>2</sup>	
Del Norte	4	76	1981
Humboldt	28	14,000 <sup>a</sup>	1989
Mendocino	26	6,300	1989
Sonoma	28	14,000	1980
Marin	28	16,000 <sup>b</sup>	1980
San Francisco	28	3,200	1971
San Mateo	28	6,000	1971
Santa Cruz	27	340	1971
Monterey	23	150	1988
San Luis Obispo	25	1,600	1989
Santa Barbara	20	4,100 <sup>c</sup>	1985
Ventura	17	309	1980
Los Angeles	19	131	1972
Orange	21	111	1980
San Diego	12	150	1985

<sup>1</sup> Number of years in which one or more samples were submitted for PSP testing.

<sup>2</sup> In micrograms toxin per 100 grams of shellfish meat. Samples are wild sea mussels unless otherwise noted.

<sup>a</sup> Sentinel sea mussels, Humboldt Bay.

<sup>b</sup> Bay mussels, Tomales Bay.

<sup>c</sup> Bay mussels, Santa Barbara Channel.

## Santa Barbara County

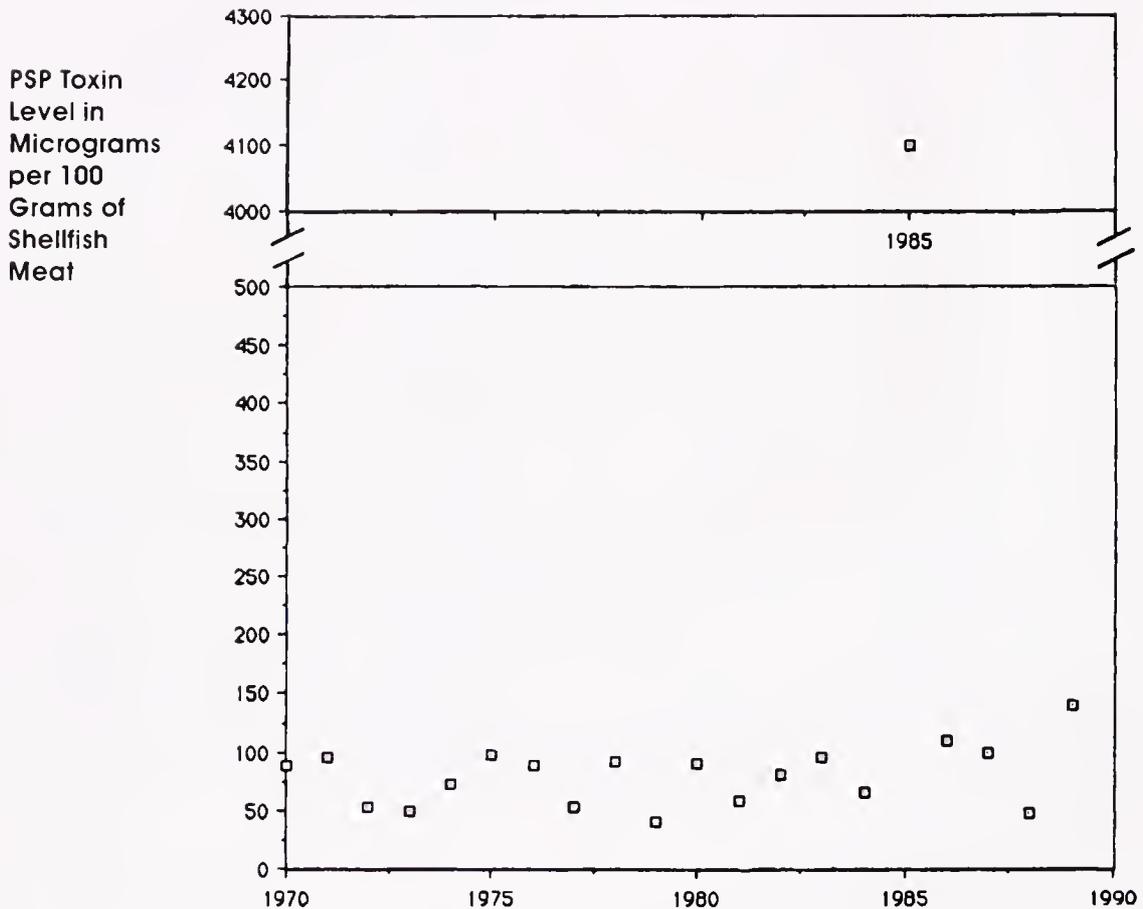


Figure 5. Highest level of PSP toxin per year reported from Santa Barbara County during the period 1970-1989

correlations between periods of high or low PSP activity and various climatic and oceanographic conditions, e.g., El Niño events, might be useful in development of bloom prediction capabilities.

Table 12 shows the distribution of PSP cases according to the source of the shellfish causing the illness. It is of interest that Santa Cruz and Monterey counties, although relatively quiet in terms of toxic dinoflagellate activity since the monitoring program began in 1962, have had a high number of PSP illnesses and deaths. All of these incidents occurred before 1962, suggesting that there may have been some changes in toxic bloom phenomena along the coast during the past 60 years.

#### Dimensions of Toxic Blooms in California

In their description of the 1927 PSP outbreak, Meyer et al. (1928) noted that human cases occurred at various locations along a 150-mile section of coast, i.e., from Wright's Beach in Sonoma County to Pacific Grove in Monterey

County.‡ Also, in the 1927 outbreak, all cases occurred within the short time period July 14-17, indicating high levels of toxicity in shellfish along this entire section of coastline. Tests conducted at the time confirmed elevated toxin levels in mussels from numerous sites along the coast, as well as from the Farallone Islands 25 miles west of the coast. Studies of the 1927 outbreak provided the first indication that toxic blooms in California may encompass extensive sections of the coast.

Table 13 shows the northern and southern limits of ten major toxic blooms that occurred between 1962 and 1989. All of these blooms yielded toxin levels in shellfish in ex-

‡The text of the 1928 paper states that the southernmost PSP case was due to mussels collected at Pescadero, San Mateo County. However, a single case from Pacific Grove, Monterey County, is indicated on the map included as Figure 1. Because that case also is included in the later paper by Sommer and Meyer (1937), we have used Pacific Grove as the southern limit of the 1927 bloom.

## San Luis Obispo County

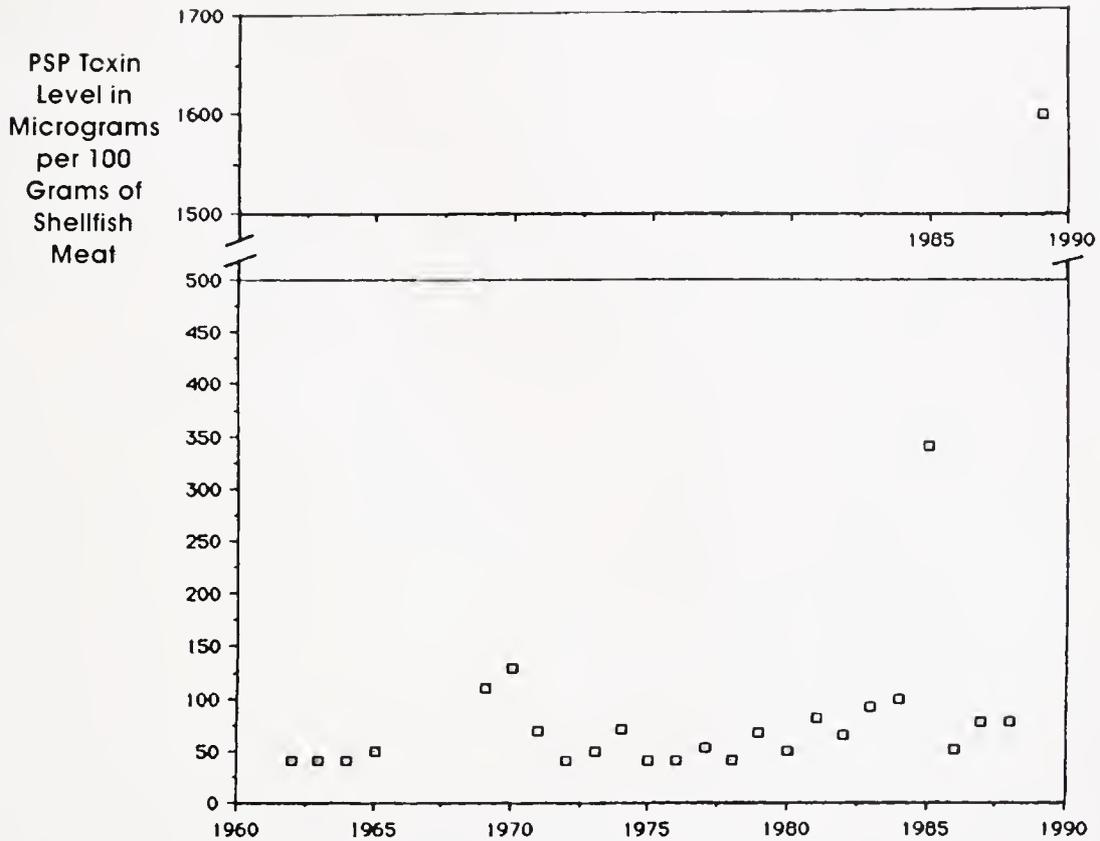


Figure 6. Highest level of PSP toxin per year reported from San Luis Obispo County during the period 1962-1989

cess of 1,000  $\mu\text{g}/100\text{ g}$ . The geographic limits are based on the apparent northern and southern points at which toxin levels in shellfish equaled or exceeded 100  $\mu\text{g}/100\text{ g}$ . Although the true dimensions are, of necessity, only estimated, information in Table 13 supports the observations of Meyer and Sommer (1937) that major blooms of toxic dinoflagellates along California's coast may extend for long distances.

#### Toxic Bloom Development

California data are insufficient to determine if major blooms grow from single locations, develop through coalescence of smaller discrete blooms (i.e., resulting from similar favorable conditions along the coast), or both. An alternative explanation is that toxic blooms develop offshore and move toward land in an already advanced stage, thereby causing rapid increases in shellfish toxicity. There is no specific evidence of this occurring from the California data, although this absence of data may be an artifact resulting from almost all sampling stations being located along the shoreline.

Better understanding of bloom development and expected variation in shellfish toxicity along sections of coastline is needed to design a more effective monitoring program. One needs to know the degree to which toxicity from one location provides information about toxin levels at nearby and distant locations along the coast. Clearly, the monitoring program should minimize the chance of blooms reaching dangerous levels without detection because sampling sites are too widely spaced.

California sampling data, to date, provide little information on variations in toxicity between adjacent sampling sites, especially during the early stages of bloom development. Nonetheless, the large size of toxic blooms which appear to be characteristic of California's coast suggest that detection should occur even with widely separated sampling sites (e.g., 10 to 20 miles) by the time a bloom has reached levels which pose a public health threat. However, as discussed later under "Dynamics of Shellfish Toxication," early detection of a developing bloom—before dangerous levels of toxin accumulate in coastal shellfish—requires an immediate increase in monitoring activities whenever low but rising levels of PSP are encountered.

## Marin County

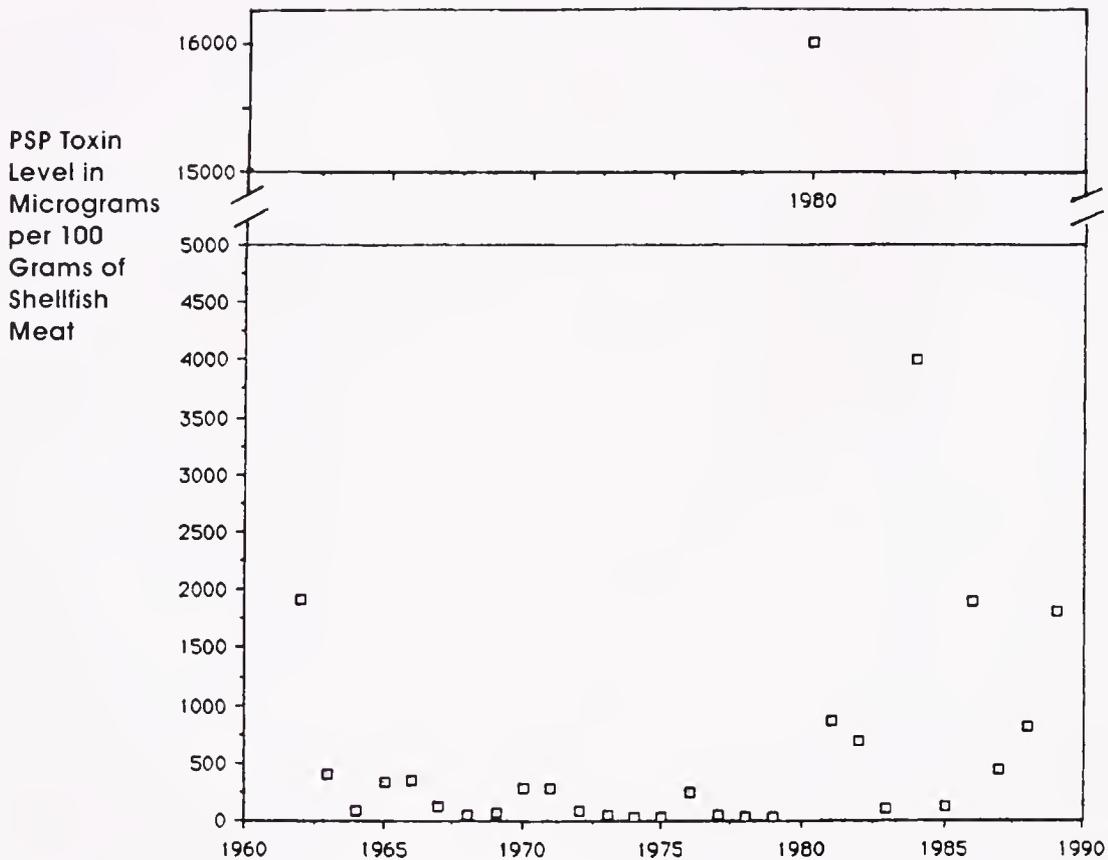


Figure 7. Highest level of PSP toxin per year reported from Marin County during the period 1962-1989

#### Duration of Toxic Blooms in California

Monitoring data associated with individual blooms not only provide information on bloom dimensions, but also on their duration. The latter, of course, is a measure of both the duration of the bloom itself and the retention time of toxin within the shellfish.

Because mussels and oysters appear to clear themselves of toxin fairly rapidly as a bloom fades, these are the best measures of bloom duration. Clams, and especially Washington clams, *Saxidomus nuttalli* (Conrad), may retain the toxin for considerable periods of time and, therefore, elevated toxin levels in clams do not necessarily indicate that a bloom is in progress.

Table 14 shows the apparent duration of the ten toxic blooms listed in Table 13 based on toxin levels in oysters and mussels. The duration of the 10 separate blooms ranged from 36 to 85 days, averaging 56 days. Of note, however, only samples with toxicity levels at or above the 100 µg level were used and, therefore, the time intervals are conservative. Also, in many cases, the amount of sampling decreased abruptly as the bloom subsided, so the time

required for shellfish to drop below the 100 µg level was not always exactly determined.

#### Dynamics of Shellfish Toxicification in California

Various investigators have noted that toxicity in bivalves can increase from safe to dangerous levels within a few days (McFarren et al. 1960). For example, Sommer and Meyer (1937) reported that toxicity in mussels from the Salmon Creek area of Sonoma County in 1932 showed a 100-fold increase in 12 days. Similarly, a study in Quebec, Canada, showed that toxicity of softshell clams (*Mya arenaria* Linnaeus) increased from 542 mouse units (~98 µg) to 26,180 mouse units (~4,712 µg) per 100 g within 6 days (Tennant et al. 1955). Further, studies in Maine showed that the blue mussel (*Mytilus edulis* Linnaeus) can increase in toxicity under optimum conditions at a rate of about 500 µg/100 g per day (Hurst and Gilfillan 1977). In the same study, the softshell clam (*Mya arenaria*) showed a maximum gain of 116 µg/100 g per day, with a daily average toxin gain of about 77 µg.

The rate of toxification is a critical factor in determining

Sonoma County

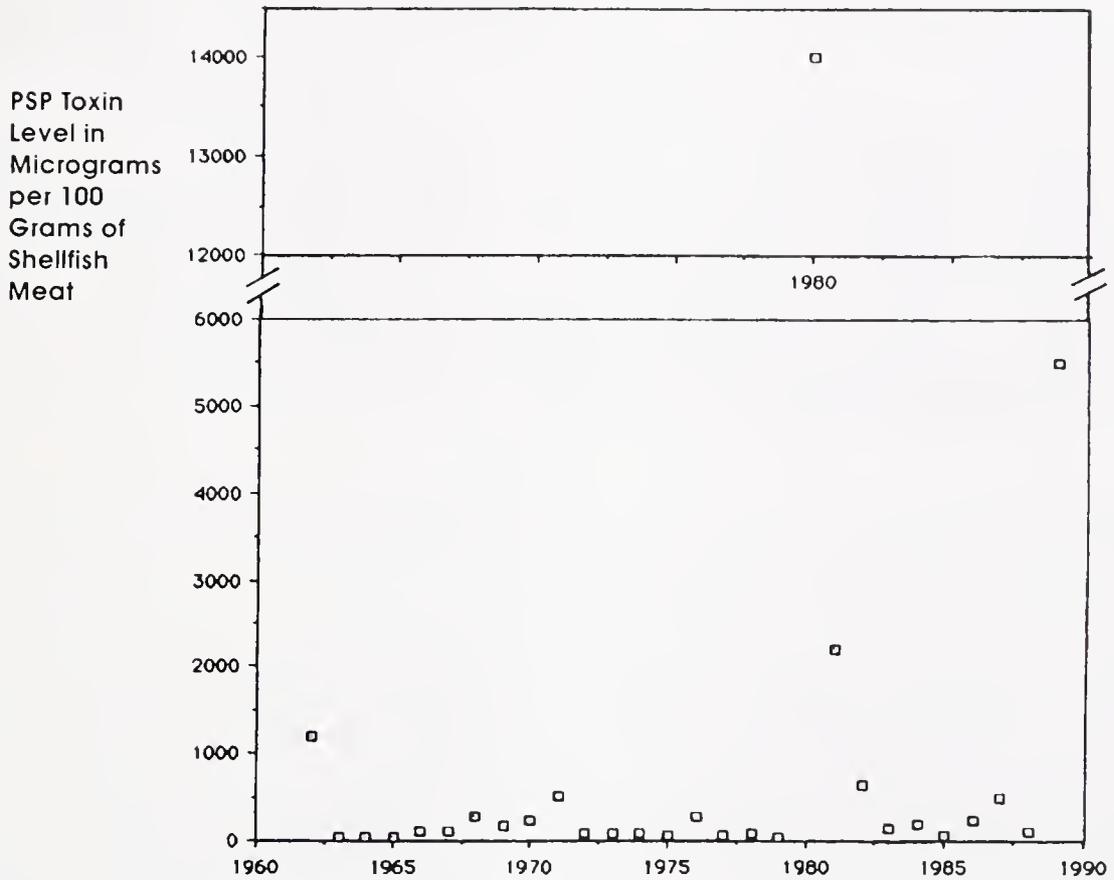


Figure 8. Highest level of PSP toxin per year reported from Sonoma County during the period 1962-1989

TABLE 12.

PSP cases in California by county, 1927-1989.\*

County	Cases <sup>1</sup>	Deaths
Del Norte	9	0
Humboldt	23	4
Mendocino	15	0
Sonoma	84	7
Marin	192	6
San Francisco	4	0
San Mateo	55	5
Santa Cruz	11	0
Monterey	103	8
Ventura	6	2
Total	502	32

<sup>1</sup> One case in 1927 and 2 cases in 1931 from unknown sources and 5 cases attributed to "Marin and Sonoma area" in 1954 are not included.

\* Counties without reported cases since 1927 are not included.

sampling frequency in a shellfish monitoring program. Monthly samples, for example, are not likely to coincide with an early period of bloom development. Biweekly samples are more useful, especially if a number of stations exist in the same region. Weekly samples, such as required by CDHS from commercial growers, are particularly helpful. Although most commercial mussel and oyster beds in California are within protected bays or estuaries, small increases in toxin levels from these areas often indicate more intense blooms in nearby ocean waters.

A third factor which may help to improve the chance of bloom detection in its early stages is described by Hurst and Gilfillan (1977). On the Maine coast, there is an early lag period when toxic blooms are "getting started" (i.e., when toxicity levels are low but detectable) and do not change greatly in intensity. This early stage may last up to two weeks before the bloom "takes off." It appears that once

TABLE 13.  
Boundaries of selected toxic dinoflagellate blooms in California, 1962–89.

Year	Months Involved	Northern Boundary	Southern Boundary	Approximate Distance (Miles) <sup>1</sup>
1962	July–August	Jenner, Sonoma County	Piegon Point, San Mateo County	113
1969	September–December	Shelter Cove, Humboldt County	Salt Point, Sonoma County	108
1970	June–August	Mussel Point, Bodega Marine Lab., Sonoma County	Pescadero Beach, San Mateo County	98
1971	July–September	Bodega Head, Sonoma County	Natural Bridges State Park, Santa Cruz County	128
1980	July–August	Timber Cove, Sonoma County (Rock Scallop)	Rockaway Beach (Pacifica), San Mateo County	92
1981	September–October	Shelter Cove, Humboldt County	Rodeo Cove, Marin County	189
1984	February–March	Mussel Point, Bodega Marine Lab., Sonoma County	Natural Bridges State Park, Santa Cruz County	130
1985	March–April	Moonstone Beach, Cambria, San Luis Obispo County	Ventura Beach, Ventura County	184
1986	June–August	Mussel Point, Bodega Marine Lab., Sonoma County	Pescadero, San Mateo County	98
1989	August–November	Trinidad Harbor, Humboldt County	Pacific Grove, Monterey County	384

<sup>1</sup> Distances are straight-line measurements following major coastal contours.

toxin levels pass the 80–100 µg/100 level, rapid increases in shellfish toxicity follow.

Toxicity data from California do not reveal such lag periods. In fact, currently available data from the CDHS monitoring program indicate that toxin levels can increase rapidly, e.g., from barely detectable levels to those capable of causing serious illness in from seven to ten days. Illustrative of this, mussels in the Santa Barbara Channel went from 43 µg/100 g on March 25, 1985, to 450 µg/100 g on April 1, to 770 µg/100 g on April 4, and finally to 4,100 µg/100 g on April 10, 1985. This represented a roughly 10-fold increase in 7 days and a 100-fold increase in 16 days. Similarly, mussels from shoreline stations at Goleta increased from 65 µg on April 1 to 2,900 on April 9; and at Gaviota, from 49 µg on April 1 to 1,800 µg on April 9. Although increases in toxicity probably are not linear, the change at the Goleta site reflected an increase of approxi-

mately 350 µg/100 g per day for eight consecutive days. A profile of the 1985 Santa Barbara bloom is shown in Figure 9.

The 1989 bloom in Northern California also provided interesting data on rates of toxification in affected shellfish. The speed at which mussels can acquire the toxin was shown dramatically in Humboldt Bay during August when samples from the Eureka-Samoa Bridge increased from undetectable levels to 5,300 µg in only 7 days, an increase of 757 µg per day. The onset and course of the bloom within Humboldt Bay is shown graphically in Figure 10.

Because of the rate at which toxin concentrations can increase in shellfish, as a bloom intensifies, it is extremely important for health officials to be alert to potential problems, and to increase sampling frequency and geographic coverage, whenever the toxin reaches detectable levels. This alert state should occur even at locations many

TABLE 14.  
Duration of nine selected toxic dinoflagellate blooms in California, 1962–89.\*

Year	Date Started	Location	Date Ended	Location	Duration in Days
1962	July 20	Drakes Estero, Marin County and Pigeon Point, San Mateo County	August 24	Jenner, Sonoma County	36
1969	September 21	Shelter Cove, Marin County	December 5	Westport, Mendocino County	76
1970	June 8	Stinson Beach, Marin County	August 7	Stinson Beach, Marin County	61
1971	July 28	Drakes Estero, Marin County	September 7	Pescadero, San Mateo County	42
1980	July 20	Drakes Estero, Marin County	August 27	Tomales Bay, Marin County	39
1981	September 9	Dillon Beach, Marin County	October 23	Rodeo Cove, Marin County	45
1984	February 21	Drakes Estero, Marin County	March 29	Drakes Bay, Marin County	38
1985	March 6	Vandenberg Air Force Base, Santa Barbara County	April 30	Goleta, Santa Barbara County	56
1986	June 4	Drakes Estero, Marin County	August 21	Graywhale Cove, San Mateo County	79
1989	August 18	Pacific Grove, Monterey County and Santa Cruz–San Mateo County Line	November 10	Sea Ranch, Sonoma County	85

\* Bloom durations are based on mussel and oyster samples with toxin levels of 100 micrograms per 100 grams of shellfish meat or greater.

## Santa Barbara Channel, 1985

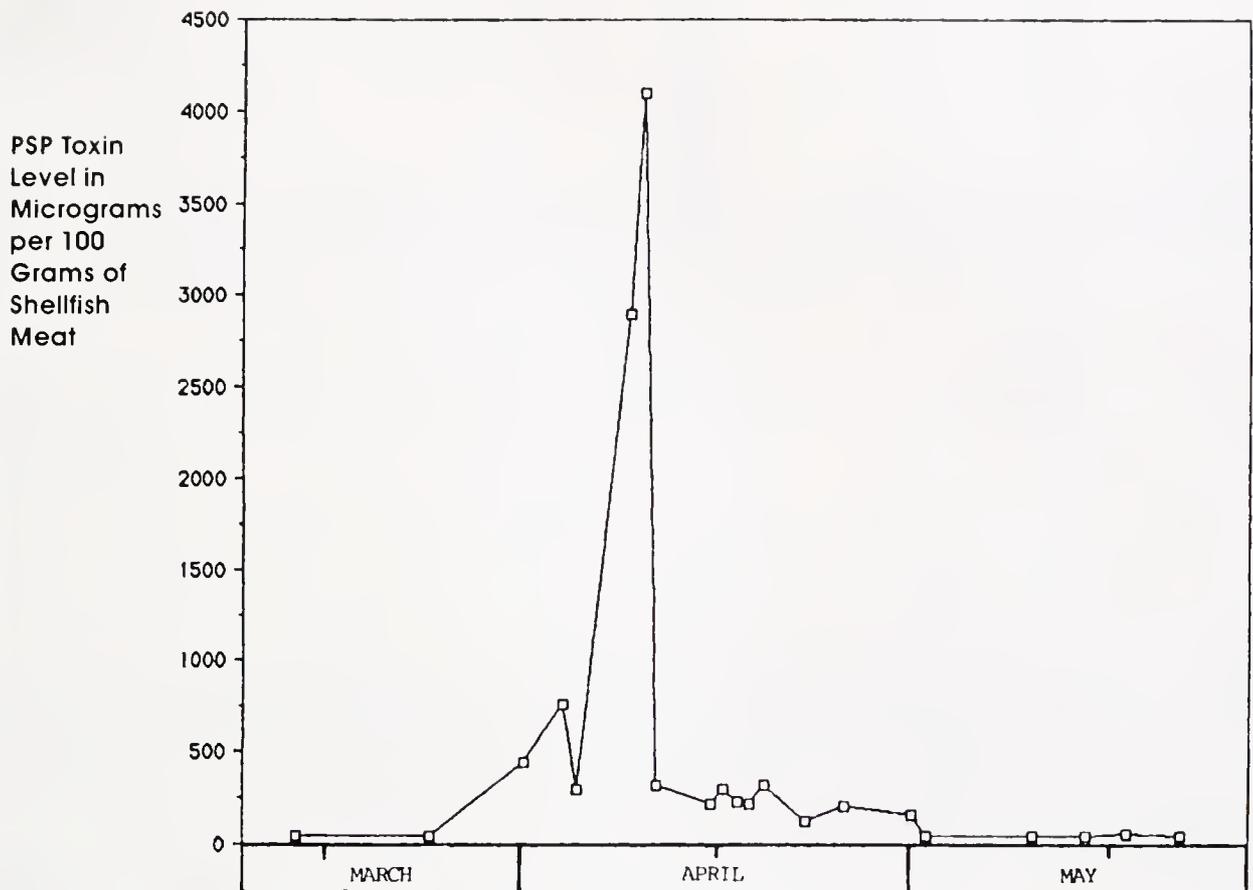


Figure 9. PSP toxin levels in mussel samples taken during the dinoflagellate bloom of 1985 in the Santa Barbara Channel, California

miles from the area of increased toxicity. For example, the CDHS and Santa Barbara County Health Department were alert to the possibility of a toxic bloom in the Santa Barbara Channel in April 1985 because of minor elevations in PSP in samples taken 2 and 3 weeks earlier at Vandenberg Air Force Base, about 70 miles away. Similarly, the rapid increase in toxicity at Humboldt Bay in September 1989 was not unexpected because of earlier PSP activity in Sonoma and Mendocino counties.

One should note that the PSP outbreak of 1980 in Marin and Sonoma counties occurred unexpectedly—the first indications being human illnesses—because of an absence of shellfish monitoring in Northern California during the preceding weeks.

Increased sampling during the August–November 1989 bloom enabled the CDHS to follow the rise and fall in shellfish toxicity in greater detail than ever before possible in California. Results of that sampling effort are shown graphically in Figures 11 and 12. In Figure 11, the sampling data are grouped and plotted according to six some-

what arbitrary geographic regions from Santa Cruz County in the south to Humboldt County in the north. This grouping allows one to visualize the northward movement of the bloom, the increase in bloom intensity as it moved northward, and the rates of increase and decline in shellfish toxicity along the selected parts of the coast. Figure 12 provides a composite of all sampling data obtained during the 1989 bloom, with the exception of duplicate counts obtained on the same date.

The northern shift in shellfish toxicity may reflect a general pattern of bloom development along this part of the coast, and, in fact, is mentioned by Sommer and Meyer (1937) with regard to the toxic blooms of 1927, 1929, and 1932 in the vicinity of San Francisco. Prediction of bloom movements would, of course, be very useful in a health protection program. More information is needed, however, before any general trends can be confirmed.

The speed at which shellfish can change from "safe" to "dangerous" is again revealed in the graphs in Figures 11 and 12. These graphs also show a fairly rapid drop in tox-

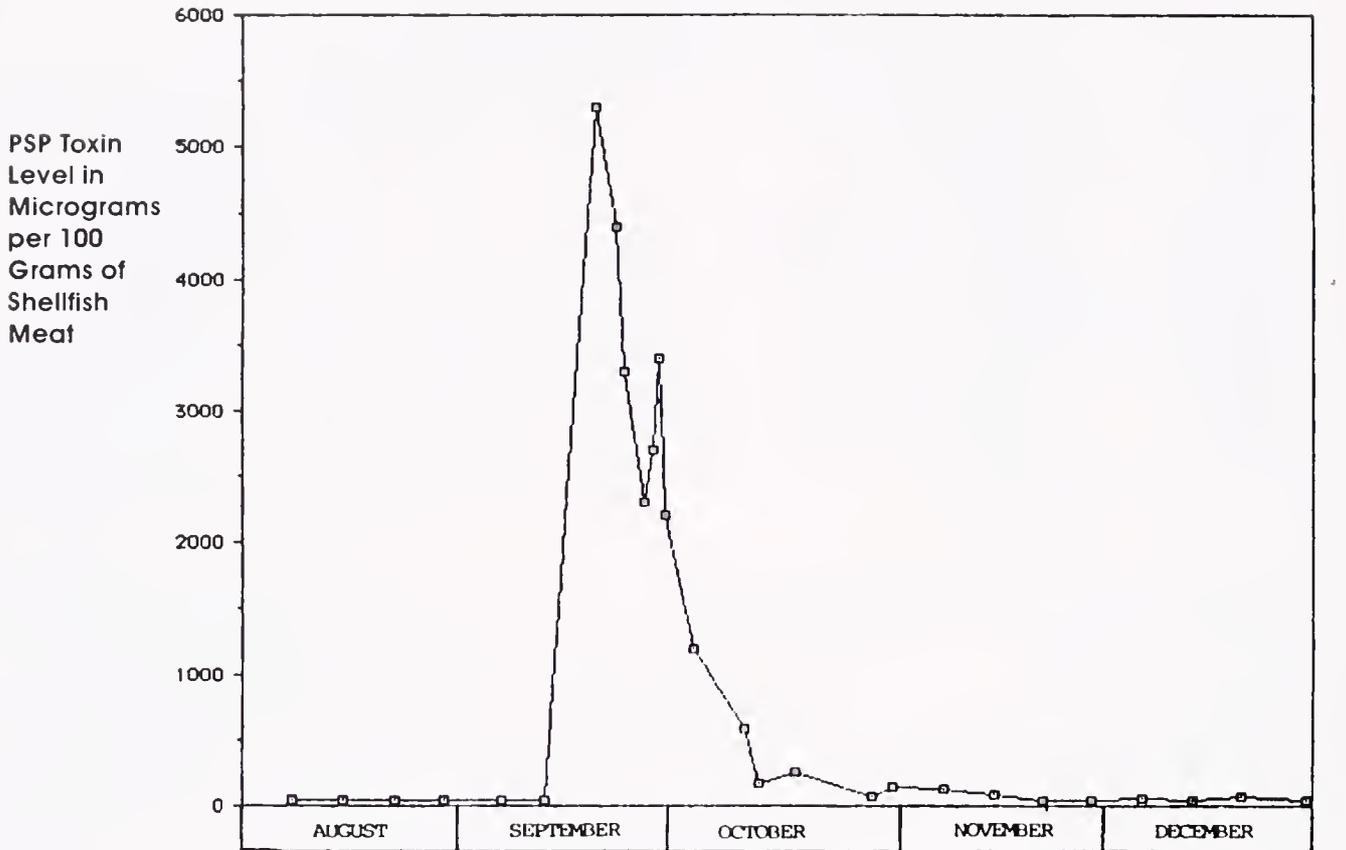


Figure 10. PSP toxin levels in mussel samples taken at the Eureka-Samoa Bridge and the Indian Island Channel, Humboldt Bay, California, during the period from August through December, 1989

icity after the peak is reached, although the drop is not as abrupt as the rise. The drop in toxicity is seen also in Figures 9 and 10 depicting the 1989 bloom in Humboldt Bay and the 1985 Santa Barbara bloom. For a public health protection program, it is important to recognize that once toxin levels begin to decline, the trend seems always to continue in a downward direction. This was true in 1989, as well as for all the major blooms in California since 1962. It would appear, therefore, that health officials need not be overly concerned that shellfish toxicity will rebound suddenly after special quarantines and public warnings are lifted.

#### Species Differences in Toxicification and Detoxification

Differences in toxicity among different kinds of shellfish living in the same locality and exposed equally to toxic blooms reflect the ability to acquire, concentrate, and/or retain the toxin. In general, the rate of toxin uptake is higher than its rate of loss; that is, toxicity can increase from low to dangerous levels in a few days, while it may take two or three weeks for the shellfish to return to safe levels after the bloom subsides. Also, detoxification rates under natural conditions (i.e., in which the bloom itself is fading) are slower than when toxic shellfish are held in dinoflagellate-free water in depuration-type tanks.

The rate of toxin loss also varies among species of shellfish. In a study of detoxification in the blue mussel (*M. edulis*), the horse mussel (*Modiolus modiolus* Linnaeus), and the softshell clam (*Mya arenaria*), Hurst and Gilfillan (1977) calculated the time required for each species to drop from 1,000 µg/100 g to less than 80 µg/100 g to be 15 days for the blue mussel, 25 days for the softshell clam, and 35 days for the horse mussel.

Sommer and Meyer (1937) noted that California sea mussels [*Mytilus californianus* (Conrad)] held in water free of dinoflagellates lost about 50 percent of their toxicity in 10 days.

Notable examples of toxin retention are found in the Alaskan butter clam, [*Saxidomus giganteus* (Deshayes)] and the Washington clam [*Saxidomus nuttalli* (Conrad)] (Shantz and Magnusson 1964; Quayle 1969). Because of this characteristic, butter clams are considered permanently toxic in some areas of the Pacific Northwest and Alaska. In these areas, the toxin is retained primarily in the neck tissues, or siphon, leading some health agencies to advise that the siphon, especially the black tip, along with the viscera, be discarded before cooking.

Washington clams, including the siphon, are generally considered safe to eat in California. However, long-term retention of the toxin was demonstrated following the 1980

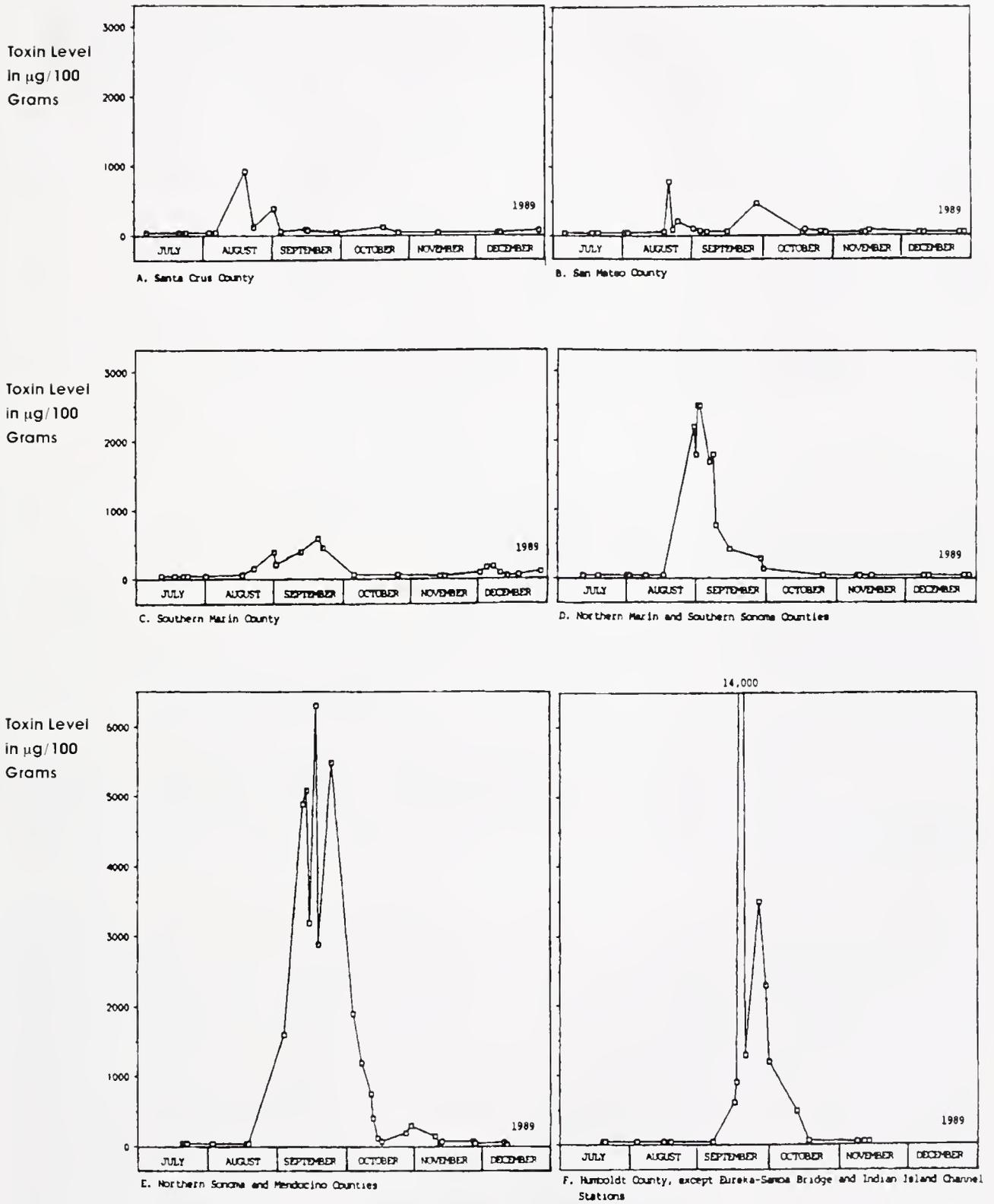


Figure 11. Paralytic shellfish poison levels in mussel samples from selected regions of the Northern California Coast during the toxic dinoflagellate bloom of August–November 1989

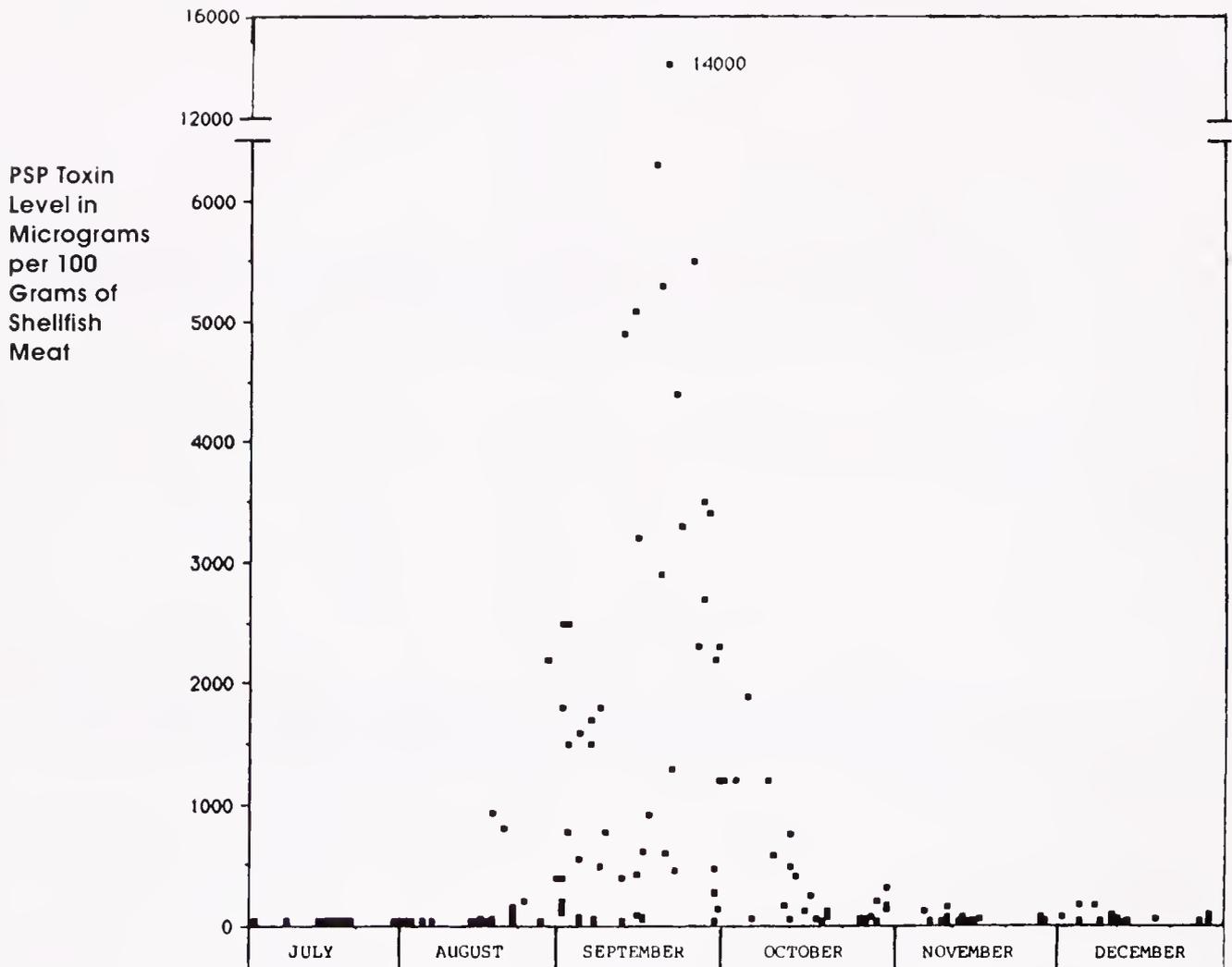


Figure 12. PSP toxin levels in mussel samples from the Northern California coast, Santa Cruz to Humboldt County, during the toxic dinoflagellate bloom of 1989

toxic bloom in Marin and Sonoma Counties. A special quarantine on the taking of Washington clams in these counties was imposed for two years, until December 1982.

In order to monitor toxin levels in Washington clams, numerous samples were taken from Bodega Harbor (Sonoma County) and Tomales Bay (Marin County) during the two-year period following the 1980 PSP outbreak. Some of these samples were divided so that the clam siphon was tested separately from the rest of the body. Results of these tests (Tables 15 and 16) confirm the persistence of the toxin and its concentration in the siphon tissues. Horseneck or gaper clams (*Schizothaerus nuttalli* (Conrad)) taken from the same sites in Bodega Harbor and Tomales Bay lost their toxicity soon after the 1980 bloom subsided (Table 17).

Data from the monitoring program also permit a comparison of toxin levels in the Pacific oyster [*Crassostrea gigas* (Thunberg)] and the bay mussel (*Mytilus edulis*)

taken from the same beds in Drakes Estero, Marin County. A comparison was made of 31 samples taken from 1984 through 1988 in which either the mussels or oysters equaled or exceeded the 80  $\mu\text{g}$  level. Of these, only one sample yielded toxin levels in oysters higher than in mussels (Table 18). In most other cases, the mussels greatly exceeded the oysters, with some mussels being five times more toxic than oysters. A similar difference between oysters and mussels was found during the 1989 toxic bloom, as shown in Table 19. These data confirm earlier observations on the propensity of mussels to acquire and concentrate the PSP toxin—an attribute which makes them the preferred indicator animal for toxic blooms and health hazard (Shumway and Hurst 1991). The wide differences in toxin levels between oysters and mussels from the same location suggest that the former may respond to concentrations of *P. catenella* by ceasing to feed or in some other manner which

TABLE 15.

PSP toxin levels in Washington clams from Bodega Harbor during and following the toxic dinoflagellate bloom of July-August 1980.<sup>a</sup>

Date	Body Less Siphon <sup>1</sup>	Siphon (Neck)	Whole Clam	Date	Body Less Siphon <sup>1</sup>	Siphon (Neck)	Whole Clam
7/30/80 <sup>b</sup>	—	—	14,000	2/6/82	71	620	—
3/3/81	220	2,800	—	3/24/82	93	780	—
3/16/81	150	1,300	—	3/24/82	55	310	—
4/16/81	—	—	1,400	6/10/82	53	320	—
8/3/81	75	950	—	6/25/82	58	140	53
9/16/81	260	1,100	—	7/9/82	<44	150	—
9/28/81	220	1,500	—	7/23/82	55	210	—
10/26/81	130	1,300	410	8/21/82	90	164	—
11/23/81	79	530	230	11/14/82	—	—	<44
11/23/81	64	340	300	11/29/82	<44	58	—
12/21/81	68	490	160				

<sup>1</sup> Body less siphon refers to all tissues of the clam, including the viscera and reproductive organs, except for the siphon.<sup>a</sup> Toxin levels in µg/100 grams of shellfish meat.<sup>b</sup> Campbell Cove.

prevents toxin uptake (Gilfillan and Hanson 1975, Gainey and Shumway 1988, Shumway et al. 1990).

#### PSP RISKS IN CALIFORNIA

In recent years, several investigators have commented on an apparent worldwide increase in shellfish toxicity problems (White 1982, WHO 1984, Maclean and White 1985, Anderson 1989, Shumway 1990); however, our data so far do not permit conclusions regarding overall increases in PSP activity along the California coast. The CDHS monitoring program has been too limited in duration and area covered to yet reveal long-term increases or cyclical patterns in toxic bloom phenomena. Increases in toxin levels noted since 1980 (Table 6) may be due simply to an increase in sampling during this period.

Similarly, it is difficult to detect any pattern in the reports of human PSP cases in California (Table 2). More

cases occurred from 1927 through 1942, than for the longer period 1943 through 1989. This drop is almost assuredly due to the imposition of the six-month annual mussel harvesting quarantine in 1942, a better understanding of the causes of PSP, better public education activities, the coastal monitoring program, and (since 1981) the requirement that growers submit weekly samples for PSP testing. There is little doubt that the CDHS PSP Prevention Program has been effective in reducing the number of illnesses and deaths from PSP. However, the risk of poisoning remains and, for several reasons, may be increasing irrespective of any absolute change in toxic dinoflagellate bloom activities off the coast.

Two factors, in particular, increase the potential danger of PSP incidents in California. These factors are the expansion of commercial shellfishing activities and the increase in sport shellfish harvesting. Commercial shellfishing, until

TABLE 16.

PSP toxin levels in Washington clams from Tomales Bay during and following the toxic dinoflagellate bloom of July-August 1980.<sup>a</sup>

Date	Body Less Siphon <sup>1</sup>	Siphon (Neck)	Whole Clam	Date	Body Less Siphon <sup>1</sup>	Siphon (Neck)	Whole Clam
8/12/80	410	4,700	—	2/21/82	<44	440	—
8/28/80	95	4,500	—	3/5/82	75	1,400	280
3/17/81	230	410	—	3/6/82	51	420	110
4/9/81	—	—	170	4/24/82	54	600	—
5/4/81	—	—	270	4/27/82	<44	110	—
5/19/81	—	—	770	5/24/82	<44	140	—
6/21/81	—	—	890	6/13/82	—	—	<44
7/1/81	—	—	76	7/7/82	<44	<44	—
12/7/81	<44	850	140	7/20/82	—	—	69
2/3/82	—	—	380	8/17/82	—	—	57

<sup>1</sup> Body less siphon refers to all tissues of the clam, including the viscera and reproductive organs, except for the siphon.<sup>a</sup> Toxin levels in µg per 100 g shellfish meat.

TABLE 17.

PSP toxin levels in horseneck or gaper clams from Bodega Harbor and Tomales Bay during and following the toxic dinoflagellate bloom of July–August 1980.<sup>a</sup>

Date	Bodega Harbor	Tomales Bay	Date	Bodega Harbor	Tomales Bay
7/30/80	7,900 <sup>b</sup>		4/10/81	<44	
8/12/80		560 <sup>c</sup>	4/16/81	<44	
		<44 <sup>d</sup>	5/7/81	<44	
8/28/80		<44	5/19/81		<44
11/19/80	<44 <sup>b</sup>		6/21/81		<44
2/3/81	<44 <sup>b</sup>		7/1/81		<44
3/3/81	52 <sup>c</sup>		8/3/81	<44	
	<44 <sup>d</sup>		9/28/81	62	
3/16/81	<44		10/26/81	<44	
3/17/81		<44	11/23/81	<44	

<sup>a</sup> Toxin levels in whole clams unless otherwise noted and in  $\mu\text{g}$  per 100 g of shellfish meat.

<sup>b</sup> Campbell Cove

<sup>c</sup> Body less Siphon

<sup>d</sup> Siphon

recently, was limited to the culture of oysters in enclosed bays and estuaries, especially Humboldt Bay, Tomales Bay, Drakes Estero, Elkhorn Slough, and Morro Bay. During the past decade, however, there has been a doubling in the number of shellfishing companies in California (i.e., from 10 in 1980 to 21 in 1988), along with changes in the types and locations of these operations.

One change, in particular, which has taken place during

the past five years has been the development of a commercial mussel industry. Although commercial mussel harvesting is still much less than that of oysters, it is increasing every year. Several companies are involved exclusively with mussel culture, and many now include mussels, along with oysters, as principal products. Because mussels take up PSP toxins more rapidly than oysters and because they tend to concentrate the toxins at higher levels, greater care must be taken to ensure a commercial product free of PSP toxins.

Another change has been the expansion of the industry from protected locations within bays and estuaries to open coastal sites where direct exposure to toxic blooms is more likely to occur. To date, open-coastal commercial shellfishing operations have been limited to the Santa Barbara Channel, although CDHS has issued permits for research and development purposes in other offshore areas in Southern California. CDHS has not approved proposed commercial shellfishing activities along the open coast of Northern California because of the much higher PSP risks there.

Increased PSP risks associated with the culturing of shellfish, and especially mussels, in open-ocean locations are countered to some extent by the general coastal shellfish monitoring program, and, more importantly, by the mandatory PSP sampling requirements for commercial harvesters. However, because of the speed at which toxin levels can increase in shellfish during a PSP bloom, CDHS has concerns about risks occurring through possible loss or delay of samples in shipment to the testing laboratory. The

TABLE 18.

PSP toxin levels in Pacific oysters and bay mussels taken at the same location and at the same time in Drakes Estero, Marin County, 1984–1988.<sup>a</sup>

Date	Location in Drakes Estero	Toxin Level		Date	Location in Drakes Estero	Toxin Level	
		Oysters	Mussels			Oysters	Mussels
2/22/84	Baries Bar 1	230	77	6/25/86	Area 15	53	190
8/15/84	Area 19	<35	80	6/25/86	Area 38	160	660
8/22/84	Area 15	44	210	6/30/86	Area 38	41	320
8/27/84	Area 15	<35	170	7/2/86	Area 13	<37	110
				8/13/86	Area 15	<41	99
7/31/85	Area 15	<43	110				
8/5/85	Area 15	<43	130	5/20/87	Area 15	42	81
9/11/85	Area 15	<43	85	7/22/87	Area 15	<41	360
				7/27/87	Area 12	<40	240
4/9/86	Area 15	46	300	7/29/87	Area 12	<40	260
4/12/86	Area 15	43	150	8/5/87	Area 38	39	92
6/4/86	Area 15	<41	170				
6/11/86	Area 15	56	550	7/27/88	Area 15	48	100
6/15/86	Area 12	480	1,200	8/1/88	Area 15	73	230
6/18/86	Area 12	69	1,900	8/5/88	Area 12	57	330
6/19/86	Area 12	66	1,100	8/9/88	Area 12	42	82
6/23/86	Area 12	46	420	12/14/88	Area 17	40	87
6/25/86	Area 12	160	920				

<sup>a</sup> Samples listed include only those in which one member of the pair equaled or exceeded 80  $\mu\text{g}/100$  grams of shellfish meat.

TABLE 19.

PSP toxin levels in Pacific oysters and bay mussels taken from area 12, Drakes Estero, Marin County, at the same time during August and September 1989.\*

Date	Toxin Level		Date	Toxin Level	
	Oysters	Mussels		Oysters	Mussels
8/24/89	110	860	9/10/89	47	470
8/28/89	70	330	9/12/89	41	270
8/29/89	220	59	9/13/89	44	220
8/30/89	58	500	9/19/89	69	260
9/5/89	59	270	9/21/89	52	210
9/7/89	49	450	9/24/89	41	150

\* Samples listed include only those in which one member of the pair equaled or exceeded 80 µg/100 grams of shellfish meat.

loss of one week's sample or failure by the grower to submit a weekly sample could create a window within which a toxic bloom might not be detected. One way to minimize this risk is to group open-coastal shellfishing allotments in the same locality, as is currently being done in the Santa Barbara Channel, so that two or more operations serve to cover each other in early detection of PSP blooms. Another solution, which, unfortunately, is not currently available, is a simpler test for PSP—that is, one which could be used by local laboratories or, possibly, by the shellfish companies themselves. If such a test were available, then it might be possible to test all shellfish harvests prior to marketing during periods of potential risk. Efforts to develop such a test need to be encouraged.

The annual mussel sport harvesting quarantine is designed to discourage the public from taking mussels during that part of the year having the highest PSP risk. However, it may not be totally effective in achieving this goal. Although an effort is made to inform the public through press advisories, posting of quarantine signs, and other public announcements, some people are not reached. These persons may not know about PSP and assume that mussels are safe at all times. Others, especially recently arrived immigrants, may not be able to read the warning signs and other public notices, even though they are printed in several languages, or they otherwise may not be able to fully appreciate the risks.

Whereas some people may not be able to fully understand the quarantine notices, others, including many long-time residents, are known to simply ignore the warnings. This is especially so because of the very sporadic nature of PSP outbreaks, both in time and location, which inevitably leads to some complacency. This is particularly true if a number of years pass without any reported PSP incidents.

Another problem inherent with an annual, preventive quarantine is that warnings are being issued even when the mussels may be safe to eat. Indeed, it is well known that some people frequently ignore the warnings and consume

mussels without ill effects. This reinforces complacency by making the quarantine seem unnecessary.

The most important characteristic of toxic dinoflagellate blooms in California from the aspect of health risk assessment is the speed at which they develop and at which affected shellfish can change from safe to dangerous food items. The wide range of human susceptibility to the PSP toxin further complicates the problem because it is difficult to assess the public health risks associated with particular toxin levels. Add to these factors the unpredictable nature of bloom events, both in time and location, and the magnitude of the CDHS PSP Prevention Program can be appreciated.

Because of the above factors, the single most important element in the State's effort to protect the shellfish consumer is the coastal PSP monitoring program. It provides information to establish emergency quarantines, issue special public warnings during the normal quarantine period, expand the mussel quarantine to include clams and other bivalve mollusks, and, when necessary, close commercial shellfishing operations. Without this monitoring program, the first indications of a toxic dinoflagellate bloom would be actual cases of PSP—an unacceptable alternative.

Effectiveness of the monitoring program depends on sampling frequency and the number and distribution of sampling stations. And while CDHS has concerns that the low sampling frequencies in certain coastal counties and the relatively long distances between some adjacent sampling stations might allow a toxic bloom to develop without early detection, recent efforts by CDHS to gain resources to increase sampling activity have improved this situation. However, these efforts need to be sustained and further improved if early detection of coastal PSP activity is to be ensured.

## CONCLUSION

PSP is a continuing and possibly increasing public health concern in California. Records of PSP illnesses since the major outbreak of 1927, along with sampling data obtained through the CDHS shellfish monitoring program since 1962, indicate that toxic dinoflagellate blooms are not an uncommon occurrence along the California coast. Because of the unpredictable appearance of toxic blooms and the speed at which dangerous levels of the PSP toxins can accumulate in exposed shellfish populations, public health protection requires a vigorous, year-round prevention effort.

The California shellfish monitoring program and testing of shellfish from commercial harvesting areas since the 1980 PSP outbreak have identified a number of PSP toxic blooms resulting in dangerously high toxin levels in coastal bivalve mollusks. Three of these recent blooms occurred during the historically "safe" nonquarantine period and posed especially high risks to sport shellfish harvesters.

Detection of these early blooms, as well as several which occurred during the quarantine period, enabled CDHS and local health agencies to issue special quarantines, close commercial harvesting areas, alert the sport shellfishing public, and take other public health protection measures. As a consequence of these actions, it is believed that numerous PSP illnesses were prevented.

The monitoring program also provides information which expands our understanding of the geography, dimensions, duration, and dynamics of toxic blooms and the toxicification of affected shellfish. This information provides a foundation upon which improvements in sampling strategies and more efficient deployment of field and laboratory resources during the course of toxic blooms can be based.

Experience in monitoring for PSP over the past several decades underscores the fact that this is a problem which

cannot be neglected by public health agencies. In California, a sustained effort, particularly in the early detection of toxic dinoflagellate blooms, is required to ensure protection of shellfish consumers. In addition, that effort ensures the safety of the State's expanding maricultural industry. Indeed, experience in other seafood industries has made clear the economically devastating effects that toxic problems can cause to such industries, and especially when they are nascent industries such as commercial shellfishing in California at this time.

In sum, we believe the California PSP Prevention Program has been effective and has provided expanded insights into this unusual malady. We think the experience gained from the California program, especially in recent years, should be useful to other states and countries confronting similar circumstances.

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## CHANGES IN THE BLOOD CHEMISTRY OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*, H. MILNE EDWARDS, 1837, OVER THE MOLT CYCLE

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**ABSTRACT** The blood chemistry of the American lobster, *Homarus americanus* Milne Edwards, was measured over the individual stages of the molt cycle. Physiological change accompanying the molt caused a significant increase in most blood constituents over the course of premolt, with a significant decline during postmolt, and followed by a gradual return to intermolt levels. The postmolt concentrations of calcium, chloride, inorganic phosphorus, magnesium, potassium, sodium, total protein, as well as osmolality, were significantly lower than during premolt. Variations in glucose could not be correlated with ecdysis. These findings, added to the already published data on lobster blood chemistry, document hemolymph constituent levels throughout the molt cycle of the American lobster.

**KEY WORDS:** lobster, *Homarus americanus*, molt cycle, blood chemistry

### INTRODUCTION

The American lobster, *Homarus americanus*, is an important commercial resource along the northeast coast of the United States and is often found in coastal waters adjacent to heavily industrialized areas. It is important, therefore, to determine how environmentally-induced stress affects this species. Such research requires knowledge of normal physiological parameters of lobsters.

One important measure of physiological well-being is the study of blood constituents. Physiological adaptations of fish to environmental stress are reflected in serum composition (Warner and Williams 1977, Wedemeyer and Yasutake 1977); both serum osmolality and blood ion profiles have been useful indicators of sublethal stress in marine invertebrates (Thurberg et al. 1973, Pritchard 1979). Some confusion has arisen, however, over the wide range of values reported over the last 50 years for blood ion concentrations in the American lobster (Mercaldo-Allen 1990).

Normal crustacean physiology is dominated by the molt cycle and each stage is associated with changes in the composition of the blood (Burse and Lane 1971, Guderley 1977). However, research on lobster physiology, including blood studies, has suffered from uncertainty regarding the molt stage of experimental animals: many early studies relied on molt-staging methods that were both inaccurate and inconvenient. Aiken has developed precise premolt-staging techniques for the American lobster that allow the accurate identification of each stage (Aiken 1973, 1980). Blood samples drawn over the molting period can be used to show how hemolymph composition changes with molt physiology.

The development of computer-assisted automated blood

analyzers for hospital use has modernized blood chemistry research. It is now possible to conduct many different tests rapidly and efficiently on a large number of samples, using small quantities of serum (Ellsaesser and Clem 1987).

Precise methods for accurate determination of molt stage, then, combined with updated technology for blood chemistry analysis now allow molt-related changes in the hemolymph of lobsters to be carefully documented. These techniques, together with a review of the existing literature, provide valuable background information for future studies of lobster physiology including studies to examine effects of pollutants and environmental degradation on the health of lobster populations.

### MATERIALS AND METHODS

#### *Animal Collection*

Adult American lobsters (67.2–92.7 mm carapace length) were collected by lobster pot or otter trawl from the Charles Island (lat. 41° 11.5 N long. 73° 03.1 W) and Morningside (lat. 41° 12.0 N long. 73° 01.1 W) waters off Milford, Connecticut. The lobsters were returned to the laboratory, marked with a sphyron tag for identification and their length and sex recorded. The animals were held in the laboratory in ambient running seawater and fed a diet of chopped fish, clam, or crab daily. During the study period (March to December, 1988) salinity ranged from 24.3 to 28.6 ppt and ambient temperature varied from 3.3 to 25.5°C. The animals were exposed to a normal photoperiod for the season.

#### *Molt-staging Criteria*

The lobsters were examined several times a week to determine their molt stage using the staging methods described by Aiken (1973, 1980). Premolt stages (D0

<sup>1</sup>The use of trade names does not imply endorsement by NMFS/NOAA.

1.0–D3 5.5) were determined by microscopic examination of the pleopods, and postmolt stages (A1–C3) by carapace characteristics. Aiken (1980) provides detailed descriptions and photographs of these molt stages. Determination of the molt stage using these techniques is accomplished rapidly and inflicts little harm to the animal, even after repeated sampling. Intermolt (C4) is distinguished by using a combination of both the carapace characteristics and pleopod observations. In the Results and Discussion following, the Aiken molt stages will be found in parentheses following the terms premolt, postmolt and intermolt as appropriate.

#### Sampling Procedure

A 1-ml sample of hemolymph was obtained by inserting a glass Pasteur pipette into the sinus at the base of one of the walking legs. To insure the health of the animals, samples were taken no more frequently than every four days (Cole 1941). Since the lobsters passed through the molt cycle at varying rates, not all individuals could be sampled at each molt stage. Clots form rapidly in lobster hemolymph (Donahue 1953), and were broken up using a pipette tip before centrifuging. The samples were spun at  $13,600 \times g$  in a micro-centrifuge for 15 minutes and the serum drawn off. Any remaining clots were again broken up, the samples recentrifuged and the rest of the serum collected. Samples were frozen at  $-12^{\circ}\text{C}$  until the analyses were conducted.

#### Chemical Analysis

Osmolality was measured with an Advanced Instruments Cryomatic Osmometer<sup>1</sup> calibrated using Advanced Freezing-Point calibration standards. Sodium and potassium levels were determined with a Perkin Elmer Flame Photometer and Primary Calibrators for serum. A Baker Centrifichem blood analyzer with automated Pipettor 1000 and an Apple II computer were used to measure serum concentrations of calcium, chloride, glucose, inorganic phosphorus, magnesium, and total protein.

Calcium, glucose, inorganic phosphorus and total protein were determined using Baker Instruments Centrifichem reagent kits. Magnesium was measured using a Rapid Stat Diagnostic kit made by the Lancer Division of Sherwood Medical. A reagent kit produced by Data Medical Associates was used to determine chloride levels.

The protein-based reference materials, Standardize I and II, manufactured by American Monitor Corporation, were used to calibrate the blood analyzer for calcium, inorganic phosphorus, and total protein measurements. Standards for the glucose test were obtained from Baker Instruments. The magnesium and chloride kits provide standards. The reliability of the analyzer's results was monitored using Beckman's Decision product, a three level liquid comprehensive chemistry control serum.

#### Statistical Analysis

A mean, standard deviation and standard error was calculated for each individual molt stage and these values are displayed in Figures 1–9 for each of the blood parameters. In order to demonstrate differences in blood ion concentration before and after the molt, the blood samples for each parameter were grouped by premolt (D0 1.0–D3 5.5) and postmolt (A1–C3) and compared by analysis of variance. A mean of the individual premolt stages was calculated and compared to a mean of the individual postmolt stages ( $N =$  premolt 189; postmolt 83). The C4 intermolt was omitted since it falls into neither of these categories. The analysis of variance was conducted using SAS (Statistical Application System) computer software (SAS Institute Inc., 1985).

## RESULTS AND DISCUSSION

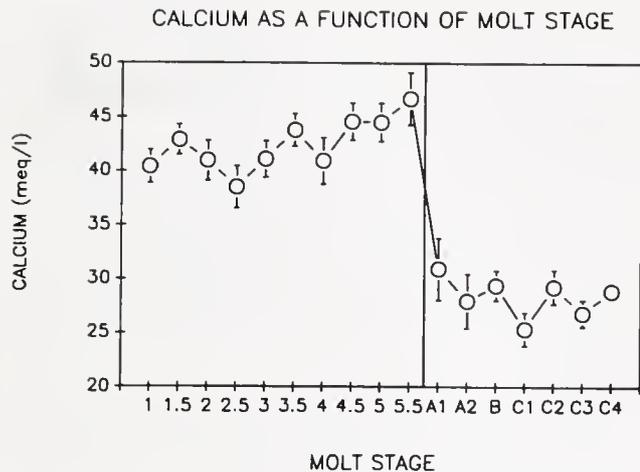
Crustaceans display certain predictable physiological variations that can be correlated directly with molt stage (McWhinnie and Mohrherr 1970, Charmantier et al. 1984a, Thuet et al. 1988). During premolt, a resorption of the exoskeleton occurs with a corresponding increase in the ion content of the blood, which provides a temporary storage site for these materials (Florkin 1960, Robertson 1960, Yamaoka and Scheer 1970, Mantel and Farmer 1983). A primary function of the blood is likely one of transport between the primary ion reservoirs, the integument and hepatopancreas (Passano 1960, Johnston and Davies 1972).

The postmolt period shows a decline in hemolymph ion concentrations as substances are withdrawn and accumulated in the cuticle and hardening exoskeleton (Florkin 1960, Hayes 1961, Price Sheets and Dendinger 1983). Uptake of water at molt increases the hemolymph volume and rapidly dilutes blood constituents (Mykles 1980, Mantel and Farmer 1983, Charmantier et al. 1984b). Water uptake occurs through the gills and body surface once the old exoskeleton begins to detach from the newly formed cuticle underneath (Mantel and Farmer 1983). Fluid absorption into the hemolymph takes place primarily through the midgut. In juvenile lobsters, seawater uptake begins an hour before molting and is completed within two hours of molt. This results in an increase in hemolymph volume from late premolt through postmolt stage A1 which decreases substantially through the remainder of postmolt (Mykles 1980).

The restoration of normal blood plasma composition at the end of postmolt requires an effective system of ionic regulation. This regulation is responsible for the observed differences in blood inorganic ions as compared to seawater and the body fluids of other marine invertebrates (Florkin 1960).

### CALCIUM

In this study, hemolymph calcium content changed over the molt cycle of *Homarus americanus* (Fig. 1). Premolt



**Figure 1.** Hemolymph calcium (meq/l) concentration measured at the individual stages of the molt cycle from D0 1.0–D3 5.5 (pre-molt) to A1–C4 (postmolt). The remaining figures use the same numerical staging system. Each point is the mean value of 7–31 lobsters; bars represent standard error.

calcium levels rose from 40.4 meq/l (D0 1.0) to a maximum of 46.7 (D3' 5.5), just prior to ecdysis (molt). Post-molt concentrations dropped off sharply to 30.9 (A1) and remained low through intermolt. The difference between pre-molt (42.4 meq/l) and postmolt (23.3 meq/l) was statistically significant at  $p < .0001$  (F value = 193.86, D.F. = 271).

Earlier studies, although lacking Aiken's precise pre-molt-staging techniques (1980), observed the connection between serum calcium and the lobster molt cycle. Donahue (1953) reported a distinct and rapid pre-molt rise in serum calcium concentrations in *Homarus americanus*: calcium increased ten hours prior to molt and reached its highest level two hours before molt. These pre-molt values were far in excess of those found during hardshell C4. Calcium values remained high through molting, but declined within two hours postmolt, reaching normal hardshell values within thirty-six hours. In another study, Castell and Budson (1974) observed that serum calcium levels reached 35 meq/l pre-molt and dropped to 25 meq/l postmolt. Charmantier et al. (1984b) also found calcium regulation in the American lobster to be affected strongly by molt stage.

*Homarus vulgaris*, the European lobster, undergoes similar molt-related changes. Glynn (1968) found serum calcium to increase markedly during early proecdysis, dropping slightly in concentration by late pre-molt. At molt, serum calcium reached a maximum, 20% above that of normal intermolt. Immediately following molt, calcium declined for ten days to a minimum value less than 50% of the intermolt level.

Travis (1955a, b) examined molting in the spiny lobster, *Panulirus argus*. A large amount of calcium was removed from the old skeleton before the molt, resulting in a marked

rise in total blood calcium. Concentrations peaked three days before ecdysis during late pre-molt. Travis found that blood calcium doubled from intermolt (22 meq/l) to pre-molt (41 meq/l). Following molt, blood calcium returned to normal intermolt levels.

Greenaway (1974a, 1985) observed that calcium transfer between exoskeleton and hemolymph in crustaceans varies seasonally because of changes in metabolism caused by the molting cycle. The pre-molt period in lobsters is characterized by increased serum calcium (Donahue 1953, Travis 1955b, Glynn 1968). In crustaceans, this increase results from resorption of considerable quantities of calcium from the exoskeleton (Travis 1955b, Bursey and Lane 1977, Mantel and Farmer 1983). Calcium is transferred into the hemolymph where it may be stored through binding with proteins produced during the molt cycle (Colvocoresses et al. 1974) or may be excreted (Passano 1960, Greenaway 1974b).

It has been suggested that elevated blood calcium late in pre-molt can be related to increased uptake of seawater just prior to and at molt (Donahue 1953, Travis 1955b), or could be a dehydration effect (Sather 1967). Although hemolymph is one of the major tissues involved in the molting process (Passano 1960), its role may be more one of calcium transport than storage (Fieber and Lutz 1985), a role that would account for the variably elevated calcium levels found in the blood prior to molt (Adams et al. 1982).

After ecdysis, the concentration of stored calcium ions in the hemolymph decreases rapidly as they are mobilized to harden the new exoskeleton (Robertson 1960, Lockwood 1967, Glynn 1968, Guderley 1977, Greenaway 1985). Calcium for the new exoskeleton comes from the old shell and from stores in the animal's tissues (Scheer 1948) and hemolymph. Uptake of calcium from the water in postmolt aquatic crustacea has been documented (Greenaway 1972). Simple diffusion through the soft integument or the gills may take place since the concentration gradient between seawater and blood calcium is favorable upon molting (Robertson 1941, Sather 1967).

Dilution by water uptake accounts for low calcium values in the blood at the time of ecdysis (Robertson 1960, Haefner 1964, Adams et al. 1982). In many crustaceans, a considerable time period is necessary to replenish blood calcium levels after molt (Henry and Kormanik 1985). During intermolt, calcium losses from the body are matched by the uptake of calcium from the water or food and the concentration of the hemolymph is relatively constant (Greenaway 1985).

#### TOTAL PROTEIN

Protein levels also changed significantly over the molting cycle (Fig. 2). During pre-molt, values rose gradually, with an unexplained decline to 7.5 g/dl (D0 2.5), before peaking at 11.4 g/dl (D1 4.0). Thereafter, levels

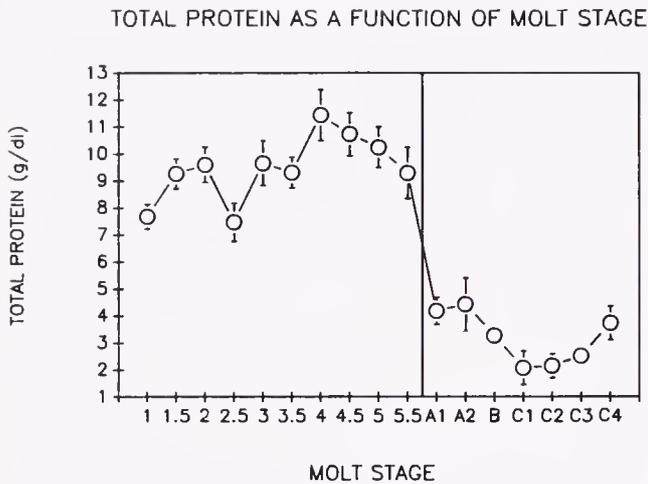


Figure 2. Hemolymph total protein (g/dl) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7-31 lobsters; bars represent standard error.

dropped off slightly until ecdysis, then declined sharply to 4.2 g/dl postmolt (A1). Values remained low through early intermolt. The premolt mean (9.53 g/dl) was significantly higher than postmolt (3.14 g/dl) at  $p < .0001$  ( $F$  value = 261.08, D.F. = 271).

Molting is one of the most important factors affecting total protein concentration in crustacean hemolymph (Djangmah 1970, Depledge and Bjerregaard 1989). Barlow and Ridgeway (1969), in their measurements of total serum protein in the American lobster, observed a high of 6.5 mg/dl during premolt before a postmolt (stage A) decline to 1.7 g/dl, with intermolt animals averaging 2.5 g/dl. Stewart and Li (1969) confirm this serum protein pattern in relation to ecdysis; they found that serum protein concentrations for "soft-shelled" were approximately 55% of the values for "old-shelled" lobsters sampled at the same time from the same location.

This pattern of serum protein change during the molt cycle is similar in other lobster species including the European lobster, *Homarus gammarus* (Glynn 1968, Hepper 1977, Hagerman 1983), the spiny lobster, *Panulirus argus* (Travis 1955a, b) and the western rock lobster, *P. longipes* (Dall 1974).

Quantitative and qualitative differences in serum protein during the molt cycle of crustaceans reflect physiological changes throughout the body (Barlow and Ridgeway 1969). The premolt period is characterized by increased total protein concentration in the blood (Florkin 1960), even though it is a time of starvation when protein levels would be expected to fall (Robertson 1960). The increase in total protein, therefore, seems to indicate a resorption of protein from the chitin-protein complex of the old exoskeleton (Passano 1960, Barlow and Ridgeway 1969, Yamaoka and Scheer 1970).

The dramatic postmolt decline in hemolymph total protein values is due to dilution of the blood by water absorp-

tion during and following molt (Travis 1955a, b, Robertson 1960, Glynn 1968, Barlow and Ridgeway 1969, Yamaoka and Scheer 1970). Utilization of protein for chitin formation during construction of the new exoskeleton can deplete blood total protein levels (Glynn 1968, Barlow and Ridgeway 1969). Starvation over the molting period may also contribute to the loss of blood proteins (Adams et al. 1982). However, blood total protein concentrations can be affected by factors other than molting, including nutritional state, season, hypoxia and environmental fluctuations (Djangmah 1970, Hagerman 1983, Depledge and Bjerregaard 1989).

The use of serum protein concentration by other authors to distinguish between molt stages has limited applications. The variation between individual lobsters is too great at any given stage for the technique to give a precise indication of the onset of molting (Hepper 1977). As an index of the amount of muscle in a live lobster, however, serum protein has been thought to provide a rapid, non-destructive means of assessing an animal's physiological condition (Stewart et al. 1967, Stewart and Li 1969). Such assessments should be careful to consider the animal's molt stage since the effects of starvation could easily be confused with those of ecdysis (Dall 1974).

#### GLUCOSE

Sugar metabolism can be closely connected to molting since blood sugar is thought to be a precursor for chitin in laying down the new exoskeleton (Knowles and Carlisle 1956). A widespread reorganization of the cuticle occurs at ecdysis and is accompanied by considerable energy requirements. In crustaceans, the blood is more important than the hepatopancreas in carbohydrate metabolism (Johnston and Davies 1972), although changes in the hepatopancreas can alter blood glucose concentration (Passano 1960). During postmolt, the exoskeleton hardens, chitin biosynthesis takes place and the glucose concentration declines (Chang and O'Connor 1983).

Fluctuations in hemolymph glucose concentration over the entire molt cycle are shown by specific stages in Figure 3. After an unexplained decline to 3.4 mg/dl early in premolt (D0 2.0), values increased gradually to a maximum of 10.2 mg/dl (D2 4.5). Glucose levels declined to 5.4 mg/dl in early postmolt (A1) before experiencing a sharp rise to 10.6 (A2). The act of molting is energy-intensive, resulting in the mobilization from the blood of large quantities of sugar and requiring the breakdown of additional glycogen to glucose. This may account for the sudden increase in glucose observed during postmolt (A2) by Baumberger and Olmstead (1928) and in this study. An abrupt rise in glucose at ecdysis that continues through postmolt stage A has been observed in other crustaceans (Passano 1960). Glucose declined postmolt (B and C1) to low levels before a gradual increase through intermolt. The premolt mean value of 7.2 mg/dl did not differ significantly from the

## GLUCOSE AS A FUNCTION OF MOLT STAGE

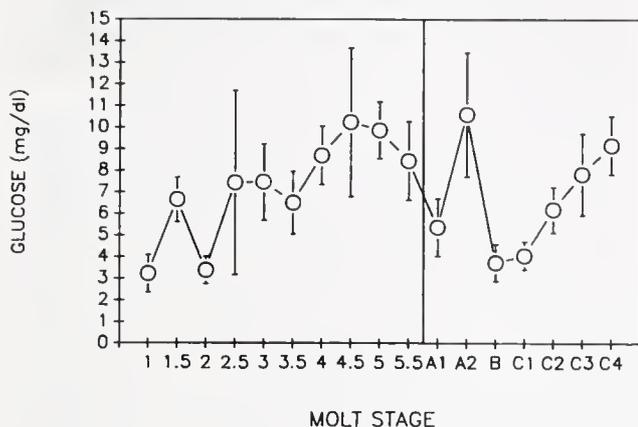


Figure 3. Hemolymph glucose (mg/dl) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7-31 lobsters; bars represent standard error.

postmolt mean value of 6.6 mg/dl (F value = 0.26, D.F. = 271).

Telford (1968a) found similar differences in glucose levels between molt stages in adult American lobsters. Mean glucose during intermolt (C4) was measured at 7.7 mg/dl. During premolt (D0-D4), levels increased to 10.5 mg/dl, 35% higher than intermolt concentrations. Following molt, glucose dropped off to 4.8 mg/dl, 30% below intermolt levels. Later in postmolt (C1-C3), glucose levels returned to normal intermolt (C4) values.

Many factors other than molt stage influence glucose levels including handling stress, hyperglycemic hormone, diet, season, temperature, circadian or tidal rhythm, stage of the reproductive cycle, physiological condition, collection, or population differences and time of sampling (Florkin 1960, Dean and Vernberg 1965a, b, Telford 1968a, b, Dall 1974, Spindler-Barth 1976, Chang and O'Connor 1983). Perhaps these factors attributed to the variability in glucose values observed in this study. Discrepancies in the literature may also be attributed to the failure of investigators to identify molt stage correctly, to the influence of ecological factors and to the diversity of analytical methods used (Jeuniaux 1970).

Variations in glucose concentration over the molt cycle are related to the changing carbohydrate needs of the animal (Telford 1968a). Glucose is a major component of circulating blood carbohydrates in crustaceans (Hohnke and Scheer 1970, Chang and O'Connor 1983). Normal blood glucose levels can be depleted through expenditure of large amounts of sugars for synthesis of new chitin and resorption of constituents from the old cuticle prior to ecdysis. Blood glucose may represent a form of transport between the glycogen of the hepatopancreas and other tissues and the sites of chitin formation (Florkin 1960). Elevated blood sugar levels are accompanied by increases in organic acids and other intermediates of glycolytic metabolism, all of

which contribute to increased osmotic pressure and facilitate water uptake during and following molt (Travis 1955a).

## INORGANIC PHOSPHORUS

Molt-related fluctuations in hemolymph inorganic phosphorus are shown in Figure 4. Concentrations increased gradually from 1.9 mg/dl (D0 1.0) to 2.9 mg/dl (D0 2.0) with an unexplained decline to 1.9 mg/dl (D0 2.5). Inorganic phosphorus reached a maximum of 3.4 mg/dl during midpre-molt (D1 4.0), before dropping steadily to 1.4 mg/dl postmolt (A1). Values remained low until returning to an intermolt (C4) level of 1.7 mg/dl. Mean values for premolt at 2.6 mg/dl were significantly higher ( $p < .0001$ ) than postmolt at 1.3 mg/dl (F value = 38.24, D.F. = 271).

In early studies with the American lobster, Hollett (1943) found that inorganic phosphorus levels increased steadily from early premolt (4.3 mg/dl) through mid-pre-molt (range 6.2-8.3 mg/dl) to a high (13.0 mg/dl) just prior to molt. The postmolt serum concentration decreased, with the lowest values obtained just after ecdysis from "papershell" animals (range 0.7-0.9). Slightly higher concentrations were detected in "rubbershell" lobsters (range 1.2-3.6). Intermolt concentrations showed some variability between animals (range 1.7-6.4).

Travis (1955b) found inorganic phosphorus levels highest during premolt in the spiny lobster, *Panulirus argus*, with a decline at the time of molt. Postmolt concentrations increased slightly (B) and then declined to intermolt values. In the lobster *Homarus vulgaris*, Glynn (1968) found a significant rise during premolt with a gradual reduction at the time of molt. Immediately after molt there was a decline, after which the concentration leveled off.

High inorganic phosphorus concentrations during premolt may result from the removal of phosphates from the old shell preparatory to molting. Inorganic phosphorus

## INORGANIC PHOSPHORUS AS A FUNCTION OF MOLT STAGE

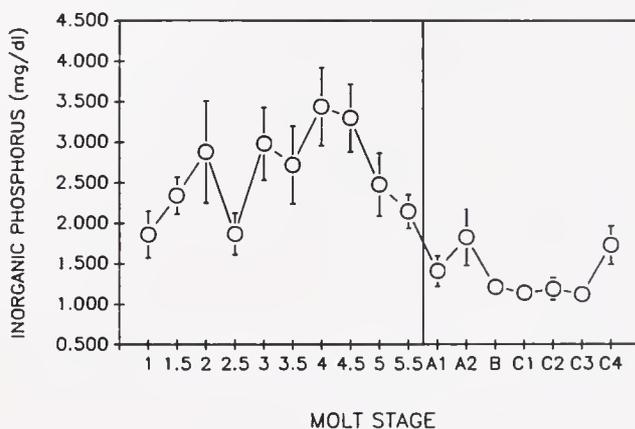


Figure 4. Hemolymph inorganic phosphorus (mg/dl) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7-31 lobsters; bars represent standard error.

levels in the blood decline through postmolt as the exoskeleton hardens (Travis 1955b). The blood is believed to furnish at least part of the phosphate necessary for the formation of the new shell (Hollett 1943), a function that would explain the low serum phosphorus concentrations after molting. The postmolt decline has also been attributed to dilution of the blood by seawater uptake (Sather 1967). Phosphate concentrations return to normal only after hardening of the exoskeleton has been completed and food consumption resumed (Travis 1955b, Sather 1967, Glynn 1968). Food is considered to be an important source of this element, and starvation can significantly decrease blood concentrations (Travis 1955b).

### MAGNESIUM

Magnesium is removed from the exoskeleton during premolt and stored in several tissues, including the hemolymph (Mantel and Farmer 1983). The role of the hemolymph in this case may be one of magnesium transport rather than storage (Price Sheets and Dendinger 1983, Fieber and Lutz 1985). Increases in magnesium during premolt are associated with resorption of salts from the cuticle in preparation for ecdysis (Robertson 1960, De Leersnyder 1967).

Changes in the magnesium composition of lobster hemolymph in this study can be followed in Figure 5. Values rose gradually through premolt peaking at 26.8 meq/l (D3 5.5). This ion remained high through early postmolt (A2) before declining to an intermolt value of 13.4. A statistically significant difference at  $p < .0188$  could be shown between mean values for premolt of 19.5 meq/l and postmolt of 21.4 meq/l (F value = 5.58, D.F. = 271).

Elevated magnesium concentrations through early postmolt have been observed in other crab and lobster species (Hagerman 1973, Spaziani et al. 1981, Towle and Mangum 1985, Wheatly 1985). In a related lobster species, *Ho-*

*marus vulgaris*, Glynn (1968) found similarly increased levels of magnesium prior to molt. During early postmolt (A), concentrations peaked (30 meq/l) before declining to a minimum value (B) (10 meq/l), followed by a return to intermolt values.

Molting in marine crustaceans occurs under conditions of elevated magnesium (Fieber and Lutz 1985). These maximal levels of serum magnesium prior to and immediately after ecdysis could be either a decrease in the efficiency of magnesium excretion from the hemolymph or an increased uptake of water during late premolt (Glynn 1968, Hagerman 1973). High levels of magnesium and calcium during premolt and early postmolt may subdue muscular activity by depressing nerve and muscle excitability. The increase in the levels of these and other blood constituents may thus contribute to the decline in activity commonly associated with the molt (Lockwood 1967, Hagerman 1973, Haefner and Van Engel 1975).

The late postmolt decline in magnesium is probably related to hardening of the exoskeleton (De Leersnyder 1967) and deposition of this ion in the cuticle (Towle and Mangum 1985, Price Sheets and Dendinger 1983).

### OSMOLALITY

Hemolymph osmotic pressure in the American lobster is affected by the molt cycle (Charmantier et al. 1984b), as was observed in this study (Fig. 6). Although there was a minor decline early in premolt (D0 1.5), values changed very little over the period, reaching a maximum level of 800 mOsm/kg (D3' 5.5). The osmolality dropped at molt, then fluctuated before returning to 735 mOsm/kg at intermolt. Mean values for premolt (768.4 mOsm/kg) were significantly higher than postmolt (743.7 mOsm/kg) at  $p < .0001$  (F value = 15.23, D.F. = 271).

The ionic composition of lobster blood conforms to that of the environment during intermolt, except under condi-

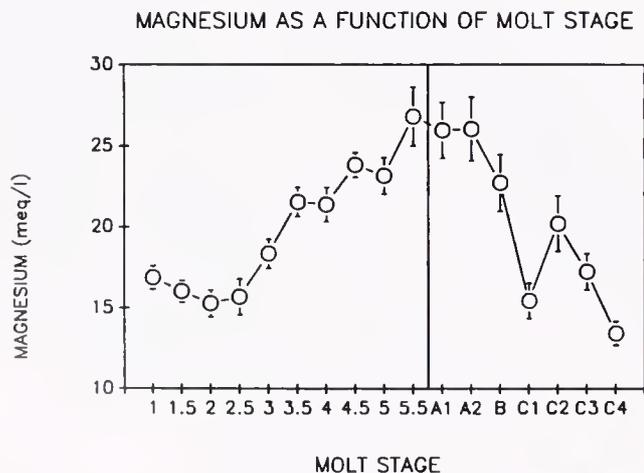


Figure 5. Hemolymph magnesium (meq/l) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7-31 lobsters; bars represent standard error.

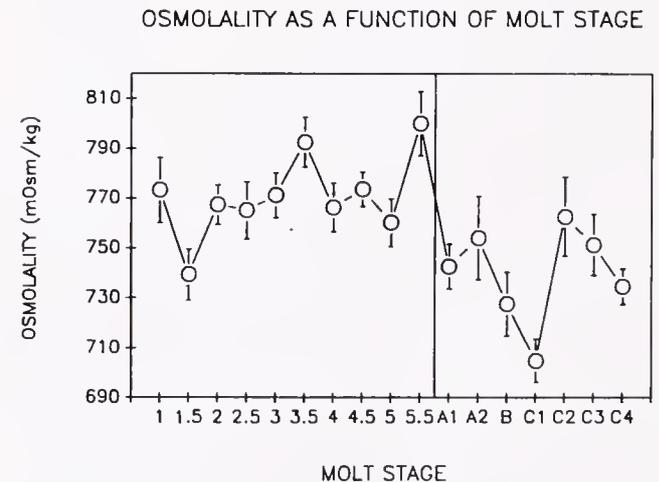


Figure 6. Hemolymph osmolality (mOsm/kg) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7-31 lobsters; bars represent standard error.

tions of low salinity (Phillips et al. 1980); this results from limited ability to osmoregulate (Dall 1970). During intermolt, the blood is maintained hyperosmotic to seawater over a salinity range of 24–26 ppt (Dall 1970, Thurberg et al. 1977, Charmantier et al. 1981) as was confirmed in this study in which the salinity ranged from 24.3 to 28.6 ppt.

In Mykles's study (1980) of juvenile American lobsters, he detected no increase from intermolt C4 (991.4 mOsm/kg) to premolt (D3) (993.7 mOsm/kg) but found a significant decline in osmolality by postmolt (A) (980.5 mOsm/kg), as was also seen in this study of adult lobsters. The postmolt decrease in osmotic pressure in *Homarus americanus* was also observed by Charmantier et al. (1988).

The slight rise in osmotic pressure during premolt can be attributed to increases in blood ionic constituents (Passano 1960, Robertson 1960, Hagerman 1973, Haefner and Van Engel 1975). This increase in internal ionic concentration establishes a gradient between the external medium and the biologically active tissues (Haefner and Van Engel 1975). An increase in free amino acid concentration may bring about a passive rise in the osmotic concentration of the blood (Robertson 1960). Conversion of hepatopancreas glycogen to glucose, known to occur during the molting cycle, could also increase osmotic pressure (Baumberger and Dill 1928, Baumberger and Olmstead 1928, Scheer 1948). The process of ecdysis, with resulting breakdown of glucose to lactic acid, could also increase the osmotic pressure (Baumberger and Olmstead 1928).

Whatever the origin, increased blood osmotic pressure is followed abruptly by an augmented water uptake into the hemolymph (Lockwood 1967, Haefner and Van Engel 1975), probably through the midgut (Mykles 1980). A hyperosmotic internal medium would favor this uptake of water at molt (Charmantier et al. 1988). Dilution of the hemolymph results in decreased osmolality following ecdysis (Travis 1955b, Glynn 1968, Mykles 1980, Charmantier et al. 1988, deFur et al. 1988). Postmolt post-larval lobsters demonstrated a lower ability to hyper-regulate than did intermolt animals (Charmantier et al. 1988).

#### POTASSIUM

Changes in hemolymph potassium over the molt cycle have not been widely studied because preliminary studies of crustacean hemolymph have revealed little or no molt-related changes (Bursey and Lane 1971, Guderley 1977, Towle and Mangum 1985). The study presented here, however, suggests that potassium metabolism over the molt cycle may be similar to that of other blood constituents. Figure 7 shows that premolt serum potassium (6 meq/l) was significantly higher ( $p < .0001$ ) than postmolt levels (4.8 meq/l) ( $F$  value = 88.04, D.F. = 271). Potassium levels remained fairly steady over premolt, peaking slightly at 6.5 meq/l (D2 4.5). Values declined slowly through molt to a low of 4.5 meq/l in postmolt (A2), remaining low thereafter until the rise in intermolt value to 5.5 meq/l.

#### POTASSIUM AS A FUNCTION OF MOLT STAGE

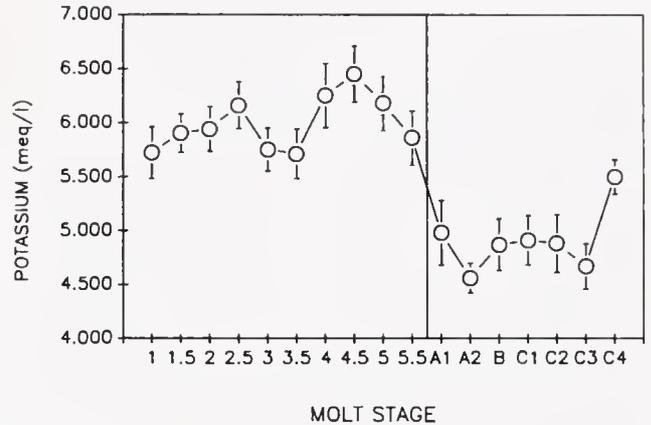


Figure 7. Hemolymph potassium (meq/l) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7–31 lobsters; bars represent standard error.

In a similar lobster species, *Homarus vulgaris*, Glynn (1968) measured serum potassium levels, finding a high of 18.7 meq/l in early premolt (D). Concentrations declined to 7 meq/l by postmolt (B).

#### SODIUM

In Figure 8, premolt sodium values were relatively steady at about 430 meq/l, declining slightly just prior to ecdysis and dropping off further during postmolt to about 417 meq/l, returning to 429 meq/l by intermolt. The premolt mean (429.1 meq/l) was significantly higher ( $p < .0001$ ) than the postmolt (416.9 meq/l) value ( $F$  value = 15.84, D.F. = 271).

Just prior to ecdysis, the surface of the old carapace cracks, resulting in increased surface permeability, which precipitates a rise in water inflow and sodium outflow by normal osmotic processes. The late premolt decline in so-

#### SODIUM AS A FUNCTION OF MOLT STAGE

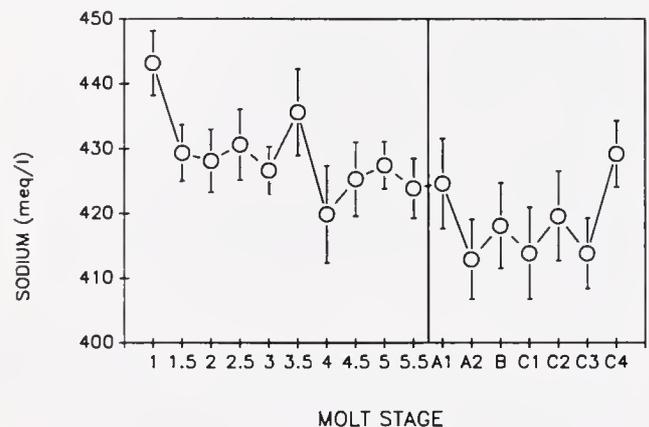


Figure 8. Hemolymph sodium (meq/l) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7–31 lobsters; bars represent standard error.

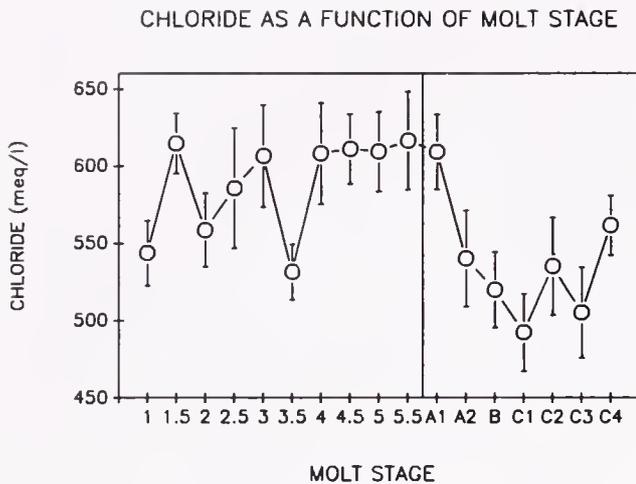


Figure 9. Hemolymph chloride (meq/l) concentration measured at the individual stages of the molt cycle. Each point is the mean value of 7–31 lobsters; bars represent standard error.

dium observed in this study may correspond to water uptake as a prelude to ecdysis (Hagerman 1973). Sodium exchanges between the blood and tissues can occur rapidly in crustaceans (Bryan 1960). In this study, sodium levels decreased in concentration during ecdysis, possibly a result of increased permeability and swelling due to water uptake (Hagerman 1973).

Studies have indicated neuroendocrine control of a sodium regulating hormone in the American lobster than can increase hemolymph sodium concentration (Charmantier et al. 1984c). Perhaps this plays a role in molt-related sodium fluctuations.

#### CHLORIDE

Chloride is one of the main osmotic effectors and is regulated in much the same way as sodium and osmotic pressure (Charmantier et al. 1984a, b). Blood chloride concentration may passively follow changes in sodium level (Lockwood 1967). Figure 9 shows hemolymph chloride concentrations throughout the molt cycle. Premolt levels fluctuated initially (D0 1.0 to D1 3.5) before stabilizing at about 611 meq/l for midpre-molt (D1 4.0) through early postmolt (A1). Chloride levels then dropped to 540 (A2), remaining low through the duration of postmolt and rising to 562 meq/l by intermolt. The premolt mean value of 589 meq/l was significantly higher ( $p < .0002$ ) than the postmolt mean of 531.9 meq/l ( $F$  value = 13.86, D.F. = 271).

Similar observations were made by Glynn (1968) for the lobster *Homarus vulgaris* who found serum chloride to vary by 2.5% from intermolt values over the molting period. Chloride concentration decreases during postmolt as a result of increased permeability and swelling. The function of chloride may differ from ecdysis to intermolt, and ion ratios during absorption and secretion can change from one molt stage to another (Ferraris et al. 1987).

#### SUMMARY

Although many researchers have investigated lobster molt physiology, the role of blood chemistry has not been sufficiently examined (Mercaldo-Allen 1990). The wide range of ion concentrations reported for the American lobster in previous studies may be attributed to natural variations resulting from the molt cycle, and to less-than-precise molt staging methods. From the very nature of the dynamic equilibrium between crustacean blood and seawater, some variation in blood composition within a single species is to be expected, particularly during the molt period (Robertson 1939). The use of refined staging methods (Aiken 1980) and updated analytical methodology has greatly enhanced both the precision and quantity of tests that can be conducted. Since only a small amount of blood is sufficient to make many different analyses, frequent withdrawal of fluids from study lobsters is possible. Such improved technology makes feasible a wide variety of measurements in which close monitoring of an individual animal is required.

Data presented here show an increase in hemolymph concentrations of calcium, chloride, inorganic phosphorus, magnesium, potassium, sodium, total protein, and osmolality over the premolt period with a decline following ecdysis. Changes in blood glucose could not be correlated with the molt cycle.

An understanding of how lobster blood physiology is affected by preparation for and recovery from ecdysis can contribute to studies of the animal's health. The study of lobster blood chemistry and the review of the literature on lobster blood studies presented here clarifies some of the complex changes associated with the molt cycle.

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## SPERMATOPHORE PRESENCE IN RELATION TO CARAPACE LENGTH FOR EASTERN BERING SEA BLUE KING CRAB (*PARALITHODES PLATYPUS*, BRANDT, 1850) AND RED KING CRAB (*P. CAMTSCHATICUS* (TILESIIUS, 1815))

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**ABSTRACT** The size at which Bering Sea red (*Paralithodes camtschaticus*) and blue (*P. platypus*) king crab produced spermatophores was examined. Over 90% of male red king crab with carapace lengths (CL) of 90–99 and 70–79 mm from Bristol Bay and Norton Sound populations respectively had spermatophores in their vas deferens. Blue king crab produced spermatophores at smaller sizes than red king crab. Over 90% of 60–69 mm CL male blue king crab from the Pribilof Islands and 50–59 mm CL males from the St. Matthew Island collections carried spermatophores.

The spermatophores from males under 100–105 mm CL from Bristol Bay red king crab and St. Matthew Island blue king crab were smaller than those of larger specimens. As males grew from 55 to ≈ 105 mm CL spermatophore diameters increased in a linear manner, but spermatophores from males 105 mm CL to the largest individuals had similar diameters.

Based on the presence of spermatophores, male red and blue king crabs reach sexual maturity at smaller sizes than those based on chela morphometry. They also reach maturity at smaller sizes than females.

**KEY WORDS:** anomura, lithodidae, spermatophore, reproduction, maturation

### INTRODUCTION

Red, *Paralithodes camtschaticus* (Tilesius), and blue, *P. platypus* Brandt, king crab are harvested commercially in the eastern Bering Sea and the Gulf of Alaska. Current regulations prohibit harvesting females and those males below a minimum size limit and also include guideline harvest quotas. Minimum legal sizes are generally set to insure that males have at least one opportunity to mate before they are harvested (North Pacific Fishery Management Council 1989). Quotas are set according to a fixed proportion of the estimated abundance of "mature" males (see Schmidt and Pengilly 1990 for an example).

The size at maturity (120 mm) is less than legal size and, for management purposes, has been estimated from the size-frequency of male red king crab found to be grasping females in a precopulatory embrace from Kodiak Island in the Gulf of Alaska (GOA). The current management plan assumes that eastern Bering Sea (EBS) male red king crab mature at 120 mm just like GOA crab. Female king crab exhibit geographically related variations in size at maturity and it is likely that males follow the same pattern. In the EBS, size at maturity also has been based on chela allometry (Wallace et al. 1949, Somerton 1980, Somerton and MacIntosh 1983). Use of captive GOA red king crab in mating experiments has provided information on the minimum size at which males can fertilize females (Powell and Nickerson 1965, Powell et al. 1973, 1974, Paul and Paul

1990a). However, similar mating studies have not been done with either red or blue king crab from the EBS. Various measures of the size at maturity are shown in comparison to legal size in Table 1.

This report supplements existing information on EBS king crab size of maturity by examining males for the presence of spermatophores. Presence of spermatophores in the vas deferens is a good indication that a male crab is sexually mature (Watson 1970), although it does not necessarily imply that an individual crab is sexually active.

During mating, king crab pass bands of spermatophores, distinct pedunculate capsules containing non-motile sperm, through an opening in the coxopodite of the 5th pereopod to the females (Marukawa 1933, Sasakawa 1971). External egg fertilization occurs in the female brood pouch formed by the broad apron like abdomen. Adiyodi (1985) following Greenwood (1972) describes the formation of spermatophores in the anomuran Pagurus as occurring in the anterior portion of the vas deferens with the formation of the peduncle occurring last in a T shaped groove. Pedunculate spermatophores are hence fully formed sexual products. In this report we consider the spermatozoa contained in pedunculate spermatophores as viable and crabs containing them to be sexually mature.

### METHODS

Blue king crabs were collected from the Pribilof and St. Matthew Island populations and red king crab from Bristol

TABLE 1.

Legal carapace length (CL mm) estimates of size at maturity for red and blue king crab males.

	Red King Crab		Blue King Crab	
	Bristol Bay	Norton Sound	Pribilof Islands	St. Matthew Island
Legal Size	137	103	137	120
Mature Size:				
Chela Allometry	103	—	108	77
Management Value	120	—	120	105

Notes: Legal sizes are converted from carapace width including spines (legal definition) to carapace length which is the commonly used scientific measurement. Management refers to sizes currently used to calculate quotas. Values determined by analysis of chela allometry are from Somerton (1980) and Somerton and MacIntosh (1983).

Bay and Norton Sound populations (Fig. 1). Collections of blue king crab were made during the months of June and July in 1982 and 1985–1989 ( $n = 521$ ). Red king crab were collected from Bristol Bay during May through July in 1982, 1985, 1987–89 ( $n = 423$ ) as well as January and February 1985 ( $n = 97$ ). Males from Norton Sound were collected in September of 1985, June 1986 and August 1988 ( $n = 237$ ). The length of each male carapace (mm) was recorded and its vas deferens placed in Bouin's fixative. Carapace length (CL) was measured from the rear of

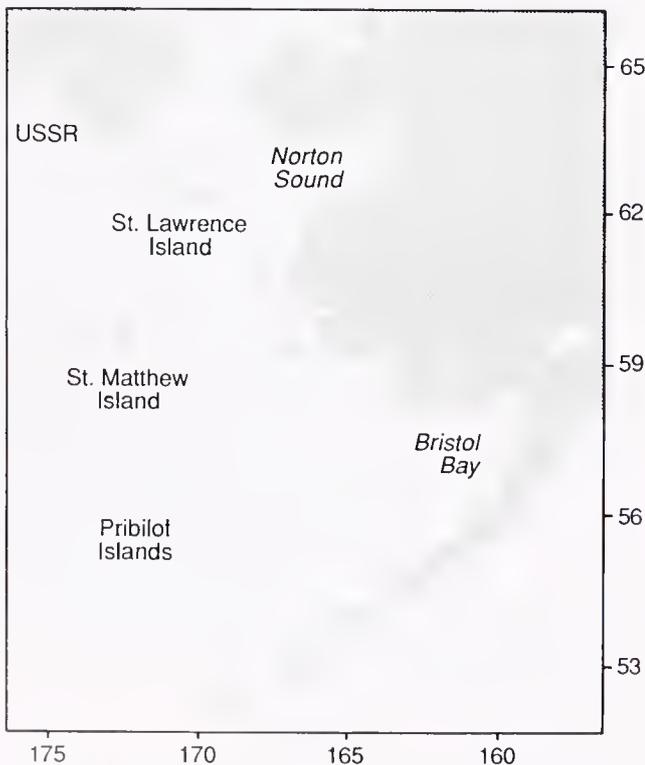


Figure 1. Map showing general collection areas for male *Paralithodes camtschaticus* and *P. platypus* for vas deferens sectioning.

the right eye notch to the median posterior of the carapace using stainless steel vernier calipers. Almost all collections were made during the course of surveys conducted by the National Marine Fisheries Service or Alaska Department of Fish and Game. We attempted to equalize sampling across the size range in each population through maintenance of cumulative tally sheets. Within this constraint, crab were sampled as available rather than randomly. The frequency of samples (Tables 2 and 3) by size is not related to the size-frequency in populations.

Standard paraffin embedding technique (Clarke 1973) was used to prepare the tissues for sectioning. Sections were cut ( $7 \mu\text{m}$ ) for staining with Ehrlich's hematoxylin and eosin y (Clarke 1973). Slides were examined under a compound microscope, ( $100\times$ ) for the presence or absence of spermatophores previously described by Marukawa (1933) for red king crab and by Sasakawa (1971) for blue king crab. The slide reader did not know the size of the crab from which the tissue was taken. Specimens from each study area were grouped in 10 mm CL increments for data presentation (Tables 2 and 3).

Diameters of spermatophores were measured for Bristol Bay red king crab and St. Matthew Island blue king crab only. Measurements were made with a calibrated ocular micrometer. These two collections were selected because they were the largest samples for each species. Slides from the other two collections did not have enough specimens with measurable spermatophores for quantitative analysis. King crab spermatophores are prolate spheroids and roughly elliptical in cross section. In this report the minor axis is considered the diameter. Only spermatophores that were positioned longitudinally and had a visible, complete peduncle were measured. In this position spermatophores had been sectioned near their centers, insuring the most accurate measurement of diameter. For each specimen, three to ten spermatophore diameters were measured, depending on availability of spermatophores in proper position in the preparation.

Our data suggested that small males produce smaller spermatophores than large males. The relationship of spermatophore diameter relative to carapace length was fit assuming a linear relationship would persist until some break point where further increase in size would not substantially affect spermatophore diameter. Break points were calculated by partitioning the data, averaged for each crab, in 5 mm steps of carapace length and then computing total sum of squares. The plot with the smallest total sum of squares was selected for presentation.

The spermatophores of both blue and red king crab look similar, and photographs from both red king crab (Marukawa 1933) and blue king crab (Sasakawa 1971) have been previously published. Inspection of our slides after the above data collection and analyses, indicated that small males had fewer spermatophores than large males. We did not, however, feel that our methods could be used to quan-

TABLE 2.

The percentage of blue king crab *Paralithodes platypus* from the Pribilof and St. Matthew Islands in the Bering Sea with spermatophores present in their vas deferens.

Carapace Length (mm)	Pribilof Islands		St. Matthew Island	
	Percent With Spermatophores	Number of Crab	Percent With Spermatophores	Number of Crab
0-29	0	16		
50-59	85	20	94	15
60-69	100	36	96	33
70-79	100	16	97	79
80-89	100	8	100	75
90-99	100	6	100	57
100-109	100	5	100	43
110-119	100	10	100	59
120-129	100	4	100	10
130-139	100	7	100	9
140-149	100	8	100	5
Total		136		385

tify such differences. Cross sections from blue king crab males of 50 and 110 mm carapace length are shown to qualitatively illustrate the differences in spermatophore abundance that we observed.

### RESULTS

Over 85% of all blue king crab males of 50-59 mm CL were producing spermatophores (Table 2). In the Pribilof Islands collection, 100% of the 60-69 mm CL group had spermatophores. In the St. Matthew Island collection 96% of that size group had spermatophores. All blue king crab larger than 79 mm CL had spermatophores. Spermatophore diameters of St. Matthew Island males tended to increase linearly with carapace length until males reached 100 mm

CL ( $Y = 1.19 \times - 32.37$ ,  $r^2 = 0.75$ ,  $n = 46$ ), while spermatophores from 100-140 mm CL males tended to have similar diameters ( $\bar{x} = 93.2 \mu\text{m}$ ,  $SE = 1.53$ ,  $n = 34$ ; Fig. 2).

Only 20% of the 40-49 mm CL red king crab males from Bristol Bay were producing spermatophores (Table 3). As carapace length increased above this size, so did the percent of males with spermatophores. A majority of males from both populations had spermatophores at 50-59 mm CL. The 90-99 mm CL group from Bristol Bay and the 70-79 mm CL group from Norton Sound were the smallest size classes in which over 90% of the specimens had spermatophores. Mean spermatophore diameters for Bristol Bay males increased linearly with carapace length through 105 mm ( $Y = 0.71 \times + 9.0$ ,  $r^2 = 0.50$ ,  $n = 51$ ), while

TABLE 3.

The percentage of red king crab *Paralithodes camtschaticus* from Bristol Bay and Norton Sound in the Bering Sea with spermatophores present in their vas deferens.

Carapace Length (mm)	Bristol Bay		Norton Sound	
	Percent With Spermatophores	Number of Crab	Percent With Spermatophores	Number of Crab
40-49	20	5		
50-59	56	16	52	25
60-69	75	37	84	50
70-79	88	52	95	49
80-89	88	99	98	49
90-99	99	101	98	44
100-109	97	93	100	14
110-119	100	58	100	6
120-129	96	26		
130-139	100	21		
140-149	100	12		
Total		520		237

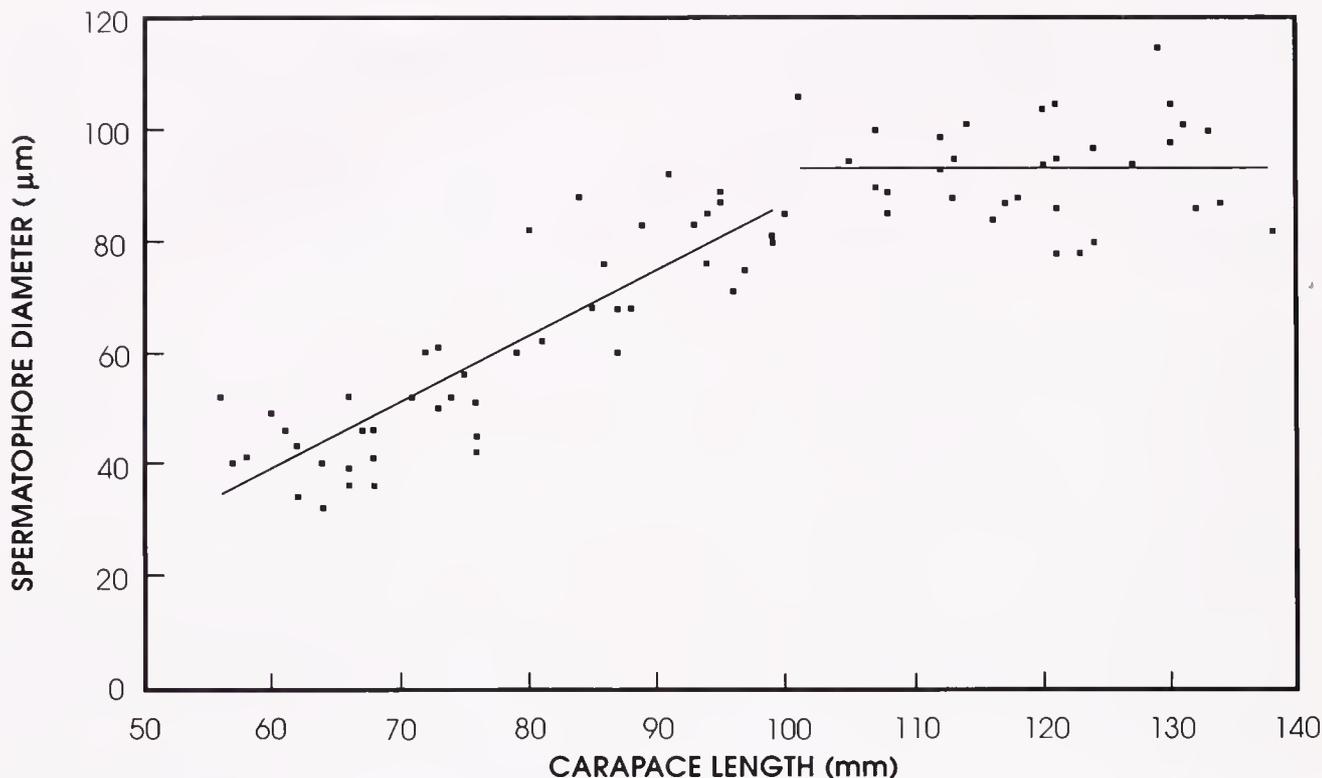


Figure 2. The relationship of mean spermatophore diameter to carapace length in *Paralithodes platypus* collected from the St. Matthew Island area.

larger males had similar spermatophore diameters ( $\bar{x} = 99.15 \mu\text{m}$ ,  $\text{SE} = 1.76$ ,  $n = 39$ ; Fig. 3).

At sizes above 105 mm CL Bristol Bay red king crab had slightly larger ( $t = 2.8$ ,  $P < 0.01$ ) spermatophores than St. Matthew Island blue king crabs, but we can not ascribe any importance to this result. Blue king crab males from St. Matthew and Pribilof populations apparently mature at smaller sizes than Bristol Bay or Norton Sound red king crab based on the percent of crabs having spermatophores. Maximum spermatophore size in St. Matthew Island blue king crab is approached more quickly with respect to carapace length than it is in Bristol Bay red king crab (slopes differ by 0.48,  $t = -3.26$ ,  $P < 0.01$ ; Figs. 2 & 3).

#### DISCUSSION

The size at maturity estimate based on the presence of spermatophores is smaller than previously published values. Based on chela morphometry, estimates of the size at which 50% of male blue king crab were mature was 108 mm CL (95% confidence interval = 83–133 mm CL) and 77 mm CL (95% confidence interval = 58–96 mm CL) for Pribilof and St. Matthew Islands respectively (Somerton and MacIntosh 1983). Although the precision of these estimates was questioned by the authors, we note that almost all males within their confidence intervals had spermatophores (Table 2).

Blue king crab males produce spermatophores at smaller sizes than that at which females first extrude eggs. Females from the Pribilof Islands and St. Matthew Island mature at 96 and 81 mm CL respectively (Somerton and MacIntosh 1983). All males above 80 mm CL carry spermatophores (Table 2) and 85% or more of the 50–59 mm CL specimens (Table 2) had spermatophores.

In the St. Matthew Island collection, maximum spermatophore diameter is reached by 100 mm CL. At smaller sizes, mean spermatophore diameter increases linearly with carapace length. Coupled with visual observations showing a paucity of spermatophores in small males (Fig. 4), the gradual increase in spermatophore size may indicate that small blue king crab males are less capable of repetitive mating as is true of red king crab (Paul and Paul 1990a). Mating experiments could address this theory and also explore the consequences of biennial spawning in females (Somerton and MacIntosh 1985, Jensen and Armstrong 1989).

The size at maturity for red king crab based on spermatophore presence is smaller than previously published values. Based on "proliferation and hypertrophy of cells lining the ductus deferens" Wallace et al. (1949) stated that most Bristol Bay male red king crab become mature at 85 to 90 mm CL. They also present data suggesting that the morphometrically based size at maturity is approximately 100 mm CL. Somerton's (1980) analysis of their data re-

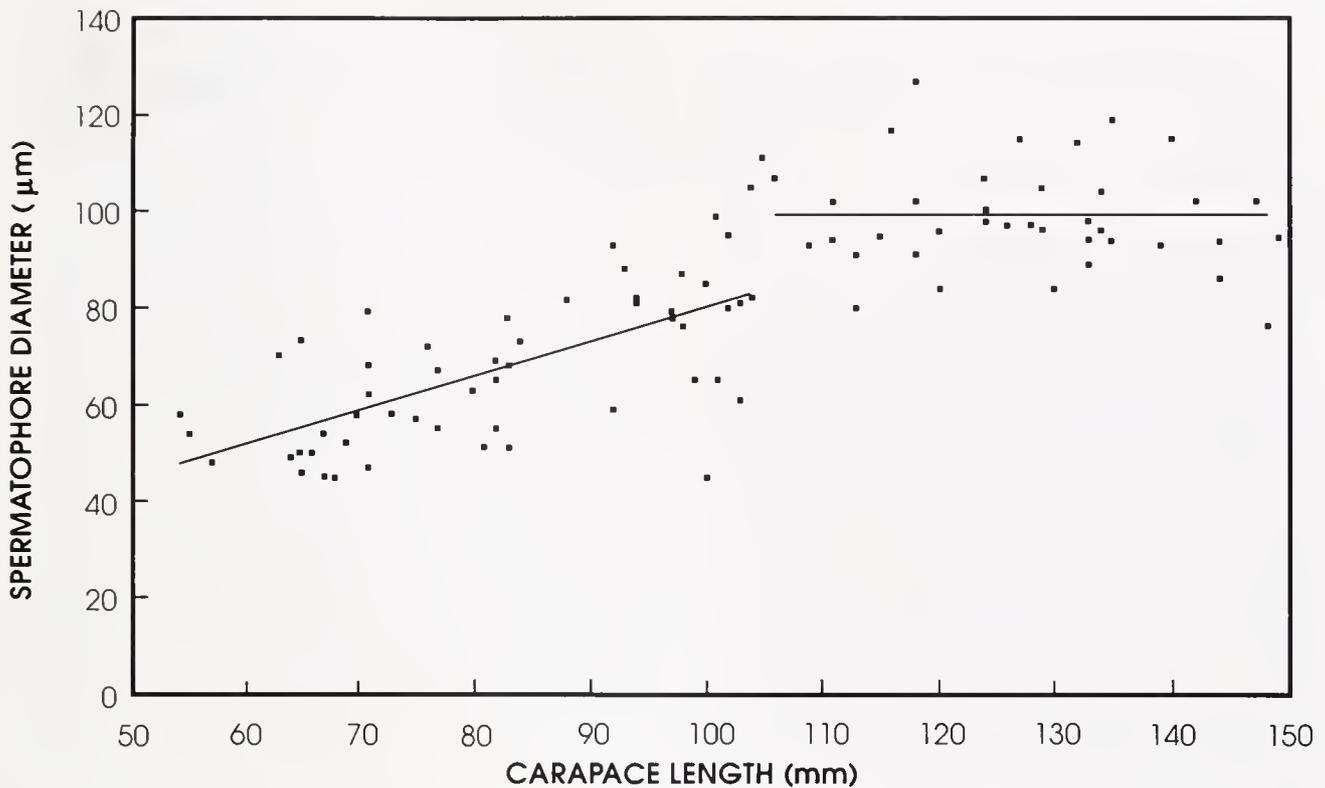


Figure 3. The relationship of mean spermatophore diameter to carapace length in *Paralithodes camtschaticus* collected from Bristol Bay.

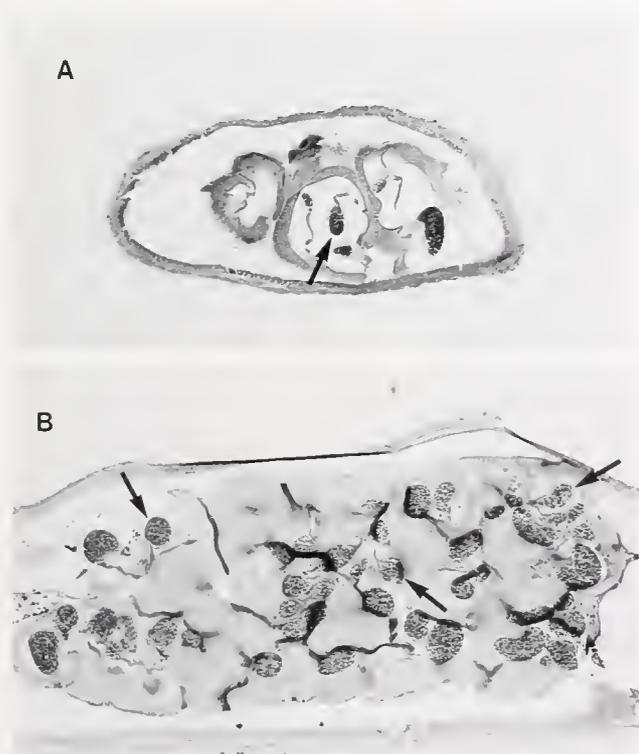


Figure 4. Relative abundance of spermatophores (arrows indicate spermatophores) in cross sections of vas deferens from St. Matthew Island blue king crab: A) 50 mm carapace length and B) 110 mm carapace length individuals.

sults in a size at maturity of 103 mm CL. Our observations show that  $\approx 50\%$  of 50–59 mm CL males and 75–84% of 60–69 mm CL males are producing spermatophores in the two populations studied.

Male red king crab also can breed at a smaller size than females (Paul and Paul, 1990a). Fifty percent of female red king crab mature after 89 and 71 mm CL in Bristol Bay and Norton Sound respectively (Otto et al. 1990), while most smaller males produce spermatophores. The above values for female size at maturity demonstrates that there is geographical variation in that aspect of reproduction. Comparison of spermatophore presence does not indicate differing sizes at maturity for males from the study areas. But, it is probable that like females there is geographic variation in the size at which males mature. Since males do not undergo pronounced morphometric changes as they mature, examining geographic variation in size at maturity is problematic, but warrants further study.

Breeding experiments suggest that small spermatophore producing red king crab that can mate have not reached full reproductive potential (Paul and Paul 1990a). Gulf of Alaska male red king crab under 90 mm CL are often incapable of fertilizing all the eggs in a clutch of more than one female. Larger males can fertilize two or more egg clutches (Paul and Paul 1990a) and some may be capable of fertilizing more than 10 females (Powell et al. 1974). Hypothetically reduced spermatophore size and abundance explains the lesser reproductive potential of small, mature males in

breeding experiments (Paul and Paul 1990a). In the GOA males in grasping pairs are typically larger than 120 mm CL (Powell and Nickerson 1965, Powell et al. 1972, Powell et al. 1974, Eldridge 1975). Females were larger than their mates in only 1 of 106 (Powell and Nickerson 1965) and 4% of 3402 (Powell et al. 1972) grasping pairs observed by SCUBA divers. Perhaps behavioral mechanisms preclude small mature males from breeding.

The measurement of spermatophores has not commonly been related to male size. This study showed that the diameter of spermatophores increased with crab size until about 105 mm which is close to the size of maturity estimated by chela morphometry. This coincidence poses the question is this simply fortuitous, or is there a relationship between the two measures of size of maturity that should be studied? Perhaps reproductive potential is determined by some critical mass of spermatophore material rather than simply the presence of sperm. In red king crab from Soviet waters, Sapelkin and Fedoseev (1986) demonstrated that there are regional variations in spermatophore size and abundance. Based on spermatophore presence, they speculated that males up to  $\approx 114$  mm CL are more active breeders than are larger crab. They also noted that not all mature males breed every season. These observations suggest that there is more that can be learned about the male role in population reproduction by the study of spermatophores.

There appear to be some seasonal aspects to sperm production with maximum spermatophore abundance occurring just before spawning (Sapelkin and Fedoseev, 1986). The seasonality of collections for our study caused some concern since Sapelkin and Fedoseev (1986) report that spermatogenesis is seasonally synchronized. Comparison of spermatophore diameters from Bristol Bay males larger than 105 mm CL did not, however, show differences between winter (January and February,  $\bar{x} = 99.7$ , SE = 2.0, n = 31) and summer ( $\bar{x} = 97.1$ , SE = 3.9, n = 8) collections. But, future comparisons of spermatophore size or abundance should be carried out with specimens captured during the period just prior to spawning.

Obviously deciding on the size of maturity for males

based solely on spermatophore presence would be imprudent. In some brachyurans, spermatophores are present in the vas deferens prior to the molt of puberty. Hartnoll (1963, 1965, 1969) and Johnson (1980) notes their occurrence in crabs smaller than those normally considered to be sexually active. But, another study of the brachyuran crab *Chionoecetes bairdi* showed that ability to produce spermatophores and to fertilize females occurred at similar sizes (Paul and Paul, 1990b). However, spermatogenesis in male king crabs may occur at a smaller size than the size of males able to mate as is true of the American lobster *Homarus americanus* (Aiken and Waddy 1980). Spermatophore presence indicates physiological maturity but perhaps not the ability to service females which may be defined as functional maturity. Experiments need to be done to see if the smaller spermatophore producing king crab can breed.

The relative abundance of mature males by size group, differential distribution of males and females and behavioral factors all probably affect the size frequency of males that actually breed in a given spawning season. Controlled experiments and more in situ observations are necessary to fully understand maturity in these two species.

#### ACKNOWLEDGMENTS

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## MALE SNOW CRAB, *CHIONOECETES OPILIO* (FABRICIUS, 1788), WEIGHT-WIDTH RELATIONSHIPS: AN EXERCISE IN MULTI-SOURCE REGRESSION

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**ABSTRACT** From August to October 1989, 710 legal-sized male snow crab, *Chionoecetes opilio*, from the east coast of Newfoundland were sampled to determine what effect shell condition had on live weight and by inference, yield. Results demonstrate that in undamaged, live individuals shell condition has little effect on weight. The implication of this information to the processing and harvesting sectors of the industry in Newfoundland is that carefully handled soft-shell crabs can be sold for the same price as hard-shelled individuals, despite reduced meat yield and quality attendant with processing soft-shelled crabs. An alternative harvesting strategy aimed at avoiding soft-shelled crabs would involve changing the supplementary fishing season from fall to spring, thereby avoiding the period of highest soft-shell crab abundance.

**KEY WORDS:** snow crab, *Chionoecetes opilio*, weight-width relationships

### INTRODUCTION

The snow crab, *Chionoecetes opilio* fishery began on the northeast coast of Newfoundland in 1968. As in any operation relying on labour-intensive processing methodologies, profitability is dependent on price, productivity, and yield from the raw product.

In most areas of Newfoundland the snow crab fishery is prosecuted from mid-April until either area quotas have been caught, or until the end of November when the fishing season ends.

In most commercial fishing areas the fishery is divided into two components; a "full-time" fleet, and a "supplementary" fleet. While the full-time fleet consists of large vessels (15–20 m) fishing 800 "Japanese-style" conical traps, the supplementary fleet consists of vessels ranging from 9.5–20 m restricted to 150 traps. While fulltime vessels are restricted to offshore areas, supplementary ones are limited to designated inshore areas. A further division of the two fleets is accomplished by the imposition of staggered fishing seasons. While the fulltime quota is usually completely harvested by late June, supplementary fisheries in most management areas do not begin until mid-September.

During July–October most areas along the coast have a high incidence of newly-molted, soft-shelled snow crab in commercial catches. Until 1986, this constituted a serious nuisance effect but a strictly enforced regulation prohibiting the landing of soft-shelled crabs, effectively prevented the problem from developing into a major concern to the processing sector.

In 1986 the regulation that had effectively prevented the landing of soft-shelled crab was struck down due to the subjective nature of the legal definition of soft-shelled crab. Although an attempt to devise a tool that will provide an

objective means of distinguishing between hard and soft-shelled has had promising results, (Foyle et al. 1989) there is at present no regulatory means of preventing the landing of this low-yield poor-quality product. This lack of enforced quality control has been particularly evident in the supplementary fisheries which coincide with the period of peak abundance of soft-shelled crabs. At-sea culling of soft-shell crabs often results in discard rate in excess of 70% with attendant high mortality, while dumping of dead crabs by processors frequently accounts for 50–60% of individual fishermen's landings (Taylor and O'Keefe 1989).

The severity of the soft-shell problem can be demonstrated from late-season plant sampling conducted during the fall of 1989. Catches of 5 fishermen landing at 3 processing facilities were chosen randomly and sampled for shell hardness and size composition. Percentage soft ranged from 9–29%, despite a so-called ban on soft-shell imposed by the processors and years of educational workshops outlining the disadvantages in landing these animals.

During the 1990 supplementary fishery, fishermen were accompanied by departmental Fishery Protection Officers who observed fishing operations in order to ascertain the severity of the soft shell discard problem. Seventeen crab fishing vessels from four management areas were sampled on September 20, 1990. Soft shell discards ranged from 5 to 60% on individual vessels. In terms of soft shell incidence by area, the mean ranged from 5 to 47% (G. Kelland, Acting District Protection Officer, Dept. of Fisheries and Oceans, Fisheries and Habitat Management Branch, St. John's, Newfoundland).

Processors who ultimately must bear the responsibility and indeed the cost of harvesting soft-shell crab, have long maintained that it was in the fishermen's interest to return soft-shell crab to the water. It was reasoned that fishermen

would enjoy increased benefits by harvesting these animals after they had recovered to a hard-shelled condition and the round weight per individual increased as the water absorbed following ecdysis was replaced by muscle, a period of from 2–3 months duration (Taylor et al. 1989).

Deregulation on the landing of soft-shell crab coincided with a sharp decline in resource availability (Taylor and O'Keefe 1987). Resource shortfalls, combined with high prices resulted in large quantities of soft-shell crab being accepted by processors at many Newfoundland ports (Taylor and O'Keefe 1988). This study was undertaken to determine by examining the relationship between weight and size for the different shell conditions, what effect, if any, the harvesting of soft-shell crab has on the whole weight of fishermen's landings.

## METHODS

### Sampling Procedure

During August, September and October of 1989, commercial-sized ( $\geq 95$  mm carapace width (CW)) male snow crab were sampled from commercial catches held at several processing plants along the northeast coast of Newfoundland and from a research cruise in Bonavista Bay.

During plant sampling, crabs were measured from randomly selected tote boxes that had been iced and stored in the facility's holding shed. The CW and degree of shell hardness (Taylor et al. 1989) were determined for all sampled animals, while animals with all limbs intact were weighed to the nearest 0.1 gm on a Sartorius Model U3600 balanced equipped with the MP 8-4 data input option designed to enhance accuracy in weighing live specimens.

At-sea sampling was directed exclusively at soft-shelled individuals. Specimens were obtained by means of baited traps fished during the annual time-series research cruise conducted in Bonavista Bay. Soft-shelled crabs were carefully placed in tote boxes, covered with a tarpaulin and transported to the crab processing plant in nearby Bonavista for detailed sampling and weighing.

710 legal-sized male snow crabs were sampled during the course of this study. Crabs were separated into groups based on shell condition and weight plotted against CW by group (Fig. 1). Widths ranged from 95 to 129 mm, while weights ranged from 275.0 to 1078.4 gms (Table 1).

### Statistical Analysis

The question is, then, whether the weight-carapace width relationship is independent of shell condition. The role of statistical analysis is to test whether the regression of weight on carapace width is the same for each of the three shell-condition groups, soft-shell, new/hard old/hard. This situation is, perhaps, best described as multisource regression. Recently, particularly in computer software package documentation, this has been referred to as analysis of covariance, but it differs from the analysis of covariance described in such classical statistical texts as Cochran

and Cox (1957), Ostle (1963) and Snedecor and Cochran (1967). Consequently, there has been some misunderstanding and incorrect application of statistical software packages in this context. Accordingly, the logic behind the methodology and the analysis of the data are here given in some detail.

Let  $x_{ij}$  and  $y_{ij}$  denote, respectively, the carapace width and the weight of the  $j$ th crab of the  $i$ th category, the categories being 1—soft-shell, 2—new/hard and 3—old/hard. Of the models relating weight to carapace width the two leading competitors appear to be

$$y_{ij} = a_i + b_i x_{ij} + e_{ij} \quad (\text{Model 1})$$

and

$$y_{ij} = a_i x_{ij} \eta_{ij}^{b_i} \quad (\text{Model 2})$$

or, equivalently,

$$\log(y_{ij}) = \log(a_i) + b_i \log(x_{ij}) + e_{ij}$$

where  $\log(\eta_{ij}) = e_{ij}$  and the  $e_{ij}$  are assumed to be independent random variables with zero mean and constant variance. These will be referred to as Model 1 and Model 2, respectively. It is sometimes convenient to think of  $\eta_{ij}$  as equal to  $1 + e_{ij}$  with the  $e_{ij}$  small, so that  $\log(\eta_{ij}) \approx e_{ij}$ .

To differentiate between the two models, the residual sums of squares from each was partitioned into two components, one representing the pure error, the other the lack-of-fit (see, e.g. Draper and Smith 1981). Specifically, for any value of carapace width, there is a range of weights. Regressing weight on carapace widths cannot do anything to reduce the variations between individual weights at a given width. This is the "pure error." The best tracking of the weights that can be made with carapace width as the single independent, or predictor, variable (including transformations) would pass through the mean values of the weight at each of the values of carapace width. The departure of the fitted line from these defines the "lack of fit." If the fit is good, the measure of the lack of fit should be comparable to that of the pure error. A lack of fit substantially less than the pure error is suggestive of a fit that is "too good to be true." For example, the lack of fit can be made zero by use of a sufficiently high-order polynomial in the independent variable, but such would almost certainly be meaningless.

To determine whether the data could be represented by a single regression the hypothesis that the lines were parallel ( $b_1 = b_2 = b_3$ ) was first tested. If this hypothesis can be accepted, one may then test the hypothesis of a common intercept ( $a_1 = a_2 = a_3$ , given  $b_1 = b_2 = b_3$ ). If either hypothesis is rejected, other subhypotheses may be tested to determine the most parsimonious acceptable model (see e.g. Warren 1974).

## RESULTS

Under Model 1 the lack of fit, although not large, was formally significant at the 1% level ( $F = 1.70$  on 80 and

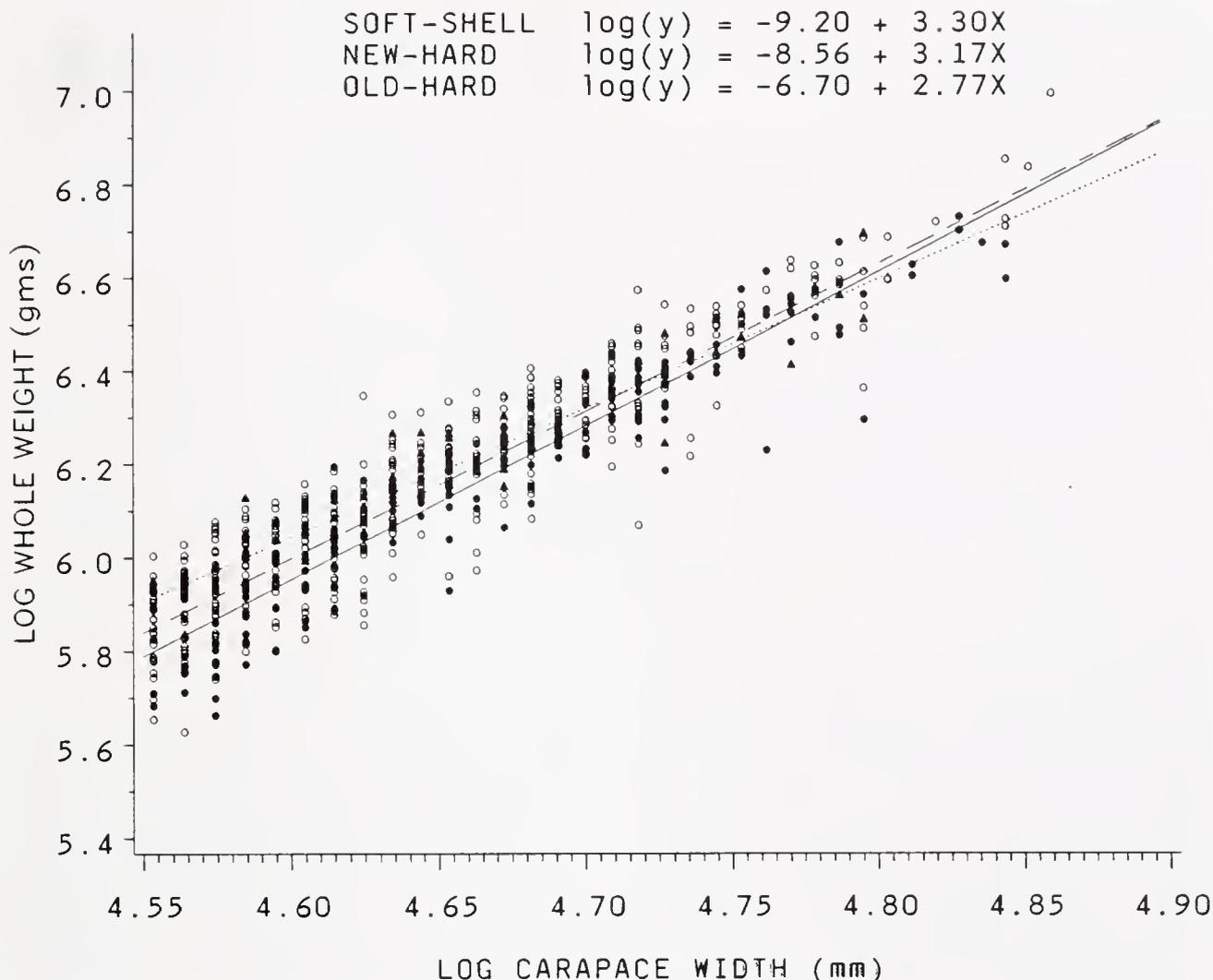


Figure 1. Plot of snow crab, *Chionoectes opilio*, whole weight (gm log<sub>e</sub>) versus carapace width (mm log<sub>e</sub>). Solid circles (solid line)—soft-shelled; open circles (dashed line)—new/hard; solid triangles (dotted lines)—old/hard.

624 d.f.) while there was no indication of lack of fit under Model 2 ( $F = 0.93$  on 80 and 624 d.f.). Accordingly, Model 2 was judged to be the more appropriate for the ensuing analysis.

The residual sums of squares under each hypothesis are given in Table 2.

A test of the hypothesis  $b_1 = b_2 = b_3$  is given by

$$F = [(4.5731 - 4.5151)/2]/[4.5141/704]$$

11.7 on 2 and 704 d.f.

The hypothesis, clearly, should be rejected. The equations of the three separate lines are:

Soft Shell:  $\log(y) = -9.2030 + 3.2949x$   
 New/Hard Shell:  $\log(y) = -8.5610 + 3.1649x$   
 Old/Hard Shell:  $\log(y) = -6.7029 + 2.7711x$

This suggests the possibility that the lines for Categories 1 and 2 might be parallel, or even coincident. Accordingly

TABLE 1.

Width-weight-shell condition for snow crabs, *Chionoectes opilio*, collected from the northeast coast of Newfoundland, August–October, 1989.

Shell Condition	No.	Width (mm)			Weight (gms)		
		Min	Mean	Max	Min	Mean	Max
Soft-shell	183	95.0	106.8	127.0	289.4	498.5	840.8
New-hard	457	95.0	104.3	129.0	275.0	476.9	1078.4
Old-hard	70	95.0	105.1	121.0	340.1	496.1	810.5

we may test the hypothesis  $b_1 = b_2$  but with  $b_3$  possibly distinct. The test statistic is

$$F = [(4.5254 - 4.5141)/(4.5141/704)] \\ = 1.7 \text{ on } 1 \text{ and } 704 \text{ d.f.}$$

which is clearly not significant at conventional levels. Some caution must be exercised here since the hypothesis being tested has been suggested by the data. Nevertheless, we may now test the hypothesis  $a_1 = a_2$ , given  $b_1 = b_2$ ; thus

$$F = [(4.6927 - 4.5254)/(4.5254/705)] \\ = 26.1 \text{ on } 1 \text{ and } 705 \text{ d.f.}$$

Clearly, this latter hypothesis must be rejected.

TABLE 2.

Residual sums of squares and degrees of freedom after fitting for prescribed models.

Model	d.f.	Residual sums of squares
Full	704	4.5141
with $b_1 = b_2 = b_3$	706	4.5731
with $b_1 = b_2 = b_3$	705	4.5254
with $a_1 = a_2$ given $b_1 = b_2$	706	4.6928

The most parsimonious acceptable fit is that of parallel but distinct lines for Categories 1 and 2 (soft shell and new/hard, respectively) with a separate line, of shallower slope, for Category 3 (old/hard). In other words, the weight-carapace width relationship is the same for soft-shell and new/hard shell crabs, but at a slightly higher level for the latter. There is a further increase in weight for old/hard shell crabs but this increase diminishes with increasing carapace width. While these differences appear to be real and make sense biologically, from a practical viewpoint, the three regression lines are very close together. The scatter and thus overlap of the individual points about the regressions (Fig. 1) is sufficiently large for there to be a high frequency of misclassification if the weight for a given carapace width were used to classify individuals as to shell type.

## DISCUSSION

Miller and Watson (1976) present a weight-width regression for *C. opilio* from the Western Gulf of St. Lawrence but do not specify the shell conditions of the animals sampled, or whether only undamaged crabs were used. Similarly, Phinney (1977) fails to report either the shell condition or degree of leg loss of the crabs sampled in deriving the regression equation he used to determine the weight-width relationship for *C. bairdi* from Alaska. It must be recognized however, that at the time these two studies were conducted, the landing of soft-shelled crab was not a problem in their study areas.

The implications of these results to the crab fishing industry are quite clear. Processors who purchase soft-shelled crabs are paying virtually the same price for them as they would for more-desirable hard-shelled individuals. In Newfoundland, fisherman are paid for the landed weight of their catches prior to butchering. Therefore the difference in yield is not evident until after processing of the catch has been completed. In purchasing soft-shelled crabs and basing payment on landed rather than butchered weight all incentive for the crab fishermen to return soft-shelled crabs to the fishing grounds is removed. It is hoped that the results of this study will encourage processors to refuse to accept these crabs on the basis of sound economics which is, as they themselves so frequently say, the bottom line. Except for the fishermen themselves, no one should be more cognizant of the magnitude of the loss to the industry. It may benefit the industry as a whole to institute a spring supplementary fishery in order to avoid the problem of soft-shell crab. The benefits in increased yield, decreased culling at-sea and dumping by processing plants should more than offset any inconvenience experienced by the industry in effecting such a change.

## ACKNOWLEDGMENT

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## SEINE HARVESTING AND FEEDING OF FORMULATED FEEDS AS NEW MANAGEMENT PRACTICES FOR POND CULTURE OF RED SWAMP CRAWFISH, *PROCAMBARUS CLARKII* (GIRARD, 1852), AND WHITE RIVER CRAWFISH, *P. ACUTUS ACUTUS* (GIRARD, 1852)<sup>1</sup>

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**ABSTRACT** Earthen ponds (0.04 ha) were initially stocked with a 1:1 ratio of male and female broodstock of *Procambarus clarkii* and *P. acutus acutus* at either 56 kg/ha or 84 kg/ha in June, 1987. No forage was planted, ponds remained filled throughout the year, and a pelleted feed was used as the primary source of nutrition. Crawfish  $\geq 75$  mm (total length) were either seine harvested (April–July) or trap harvested (April–August) during 1988 and 1989.

Yields during the first year ranged from 558 to 1365 kg/ha. Second year yields substantially increased and ranged from 1122 to 2942 kg/ha. Between 75 and 90% of the total annual harvest occurred during April, May and June. Batch harvesting was successfully achieved with seining. Total yields from trap harvested ponds were approximately  $1.9 \times$  those of seine harvested ponds. During the first harvest season mean tail meat yields ranged from 15.4 to 23.3%. Feed conversion ratios for the two production seasons ranged from 0.81 to 3.84.

**KEY WORDS:** crawfish, *Procambarus clarkii*, *P. acutus*, harvesting, feeding, management

### INTRODUCTION

Currently crawfish culture in the southern United States almost exclusively involves two species, the red swamp crawfish *Procambarus clarkii* and the white river crawfish *P. acutus acutus*. Traditional culture practices accommodate the life history of these species which inhibit regions where flooding and drought conditions alternate. In Louisiana, where most crawfish culture in the United States is practiced, initial stocking of shallow earthen ponds averaging 30 to 45 cm in depth occurs during the late spring. Ponds are then drained in late May or early June, rice *Oryza sativa* or some other forage is planted, and the pond bottom remains dry throughout the summer. The forage may or may not be harvested. Ponds are refilled during mid-September or October and the typical growing season for crawfish occurs during late fall, winter, and spring. Crawfish are trap harvested from November through June and harvests in subsequent years are generally based upon maintaining self-sustaining populations. Crawfish yields can vary from year to year and are influenced by specific environmental conditions within a particular pond, seasonal weather conditions, and management practices followed by the farmer. Annual yields in Louisiana range from approximately 560 to 1678 kg/ha (Huner 1987).

Future improvement of crawfish aquaculture is constrained by existing management techniques. For example, trap harvesting may account for 40–60% of the gross pro-

duction costs of a commercial crawfish enterprise (Cook 1981, Lawson and Wheaton 1983, Huner and Barr 1984). In addition, planting and reliance upon forage as a source of nutrition severely limits the expansion of the growing and harvest seasons.

Huner (1978) investigated the effect of food provision to supplement planted forage and interest in the evaluation of other non-traditional management practices has continued. Management practices that forego the draining of ponds and the planting of forage in favor of crawfish being exclusively fed a pelleted feed have yet to be evaluated. In laboratory and pond trials, crawfish readily eat feeds formulated specifically for crustaceans or catfish and generally respond with favorable growth (Huner and Barr 1984). Periodic batch harvesting by seine has yet to be practiced but may be a practical alternative to traditional trapping regimes.

This investigation was designed as a preliminary evaluation of the potential of new management techniques, specifically ponds filled year round, the use of pelleted feeds, and seine harvest as alternatives to draining, planting forage, and trapping, respectively. Product quality, specifically tail meat yield, tail muscle texture, and shell hardness, associated with these new practices, was monitored during a harvest season.

### METHODS

#### *Sites*

Two independent study sites were used in this investigation. Study site SFARU was located at the South Farm Aquaculture Research Unit of the Mississippi Agricultural

<sup>1</sup>Mississippi Agricultural and Forestry Experiment Station (MAFES) Publication Number J-7748.

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and Forestry Experiment Station (MAFES) on the campus of Mississippi State University, Starkville, Mississippi. At this site nine earthen ponds ranging in size from 0.04 to 0.06 ha were stocked on 16 June 1987 with 91–100% *P. clarkii* and 0–9% *P. acutus acutus* broodstock using a 1:1 male to female ratio (Table 1). Six ponds were randomly selected for stocking at 56 kg/ha and three at 84 kg/ha. No forage was planted. Ponds were supplied with well water and depths ranged from 0.61 to 1.22 m. After initial stocking, a YSI Model 57 Dissolved Oxygen Meter (Yellow Springs Instrument Company, Yellow Springs, OH) was used to determine dissolved oxygen and bottom temperature three times per day (0630, 1530, and 2400 h) from 16 May through 15 November in 1987, 1988, and 1989, and twice daily (0630, and 1530 h) from 16 November 1987 and 1988 through 15 May 1988 and 1989, respectively. Emergency aeration was provided by one ½ horsepower Air-O-Lator (Air-O-Lator, Kansas City, Missouri) in each of the nine ponds whenever the dissolved oxygen content of the pond water decreased or was expected to decrease below 3 ppm. Water pH in each pond was measured daily (1500–1600 h) and ponds were partially flushed whenever values reached or exceeded 10. Cracked corn (23 kg) was added to each pond on 18 May 1988 and 25 May 1989 to serve as a means to control rapid increases in pH levels. Diesel fuel was applied to all nine ponds on 26 May 1988 and again on 9 September 1988 at a rate of 46.7 l/ha in order to control air-breathing aquatic insects that might prey upon newly hatched juvenile crawfish.

TABLE 1.

Stocking of crayfish by pond at SFARU. R = red swamp crayfish, *Procambarus clarkii*, W = white river crayfish, *P. acutus acutus*.

Pond	Water surface area (ha)	Stocking rate (kg/ha)	Number of each species stocked
A15	0.048	55.8	33R, 2W 31R, 4W
B13	0.057	55.6	44R, 1W 40R, 5W
B16	0.065	55.7	46R, 2W 47R, 1W
A16	0.053	83.6	55R, 2W 53R, 4W
B12	0.057	83.2	59R 59R
B14	0.057	83.5	56R, 3W 58R, 1W
A13	0.053	55.5	36R, 3W 38R, 1W
A14	0.048	56.5	34R, 1W 33R, 2W
B15	0.057	55.3	37R, 4W 41R

Two groups of three ponds stocked at 56 kg/ha were randomly assigned to be either exclusively trap (A15, B13, B16) or seine harvested (A13, A14, B15). The remaining group of three ponds (A16, B12, B14), which were stocked at 84 kg/ha, were exclusively trap harvested. Pond A13 was not included in the study because no crawfish could be detected in the pond. The absence was possibly due to characteristically high pH levels recorded for this pond soon after stocking. Pond B12 was not harvested consistently after the week ending 23 April 1988 due to chronically low daily harvests. Both of these latter ponds and pond A14 were not included in the 1989 harvest. During the 1989 harvest period ponds A15 and B15 were seined harvested and the remaining four ponds continued to be trap harvested as in the previous year.

The other study site, DELTA, was located at Merigold, Mississippi (Bolivar County) and included a 1.0 ha earthen pond devoted to commercial culture. The pond contained well water and ranged in depth from 0.46 to 0.61 m. A mixture of *P. clarkii* and *P. acutus acutus* at a 1:1 sex ratio were originally stocked during May 1986 at 56 kg/ha. The pond was drained in July 1986 and rice was planted during the summer of 1987 and served as forage for most of the normal growing season. Data were collected three days per week (Thursday, Friday, and Saturday) from 8 April until 1 August 1988. Dissolved oxygen and bottom temperature were monitored twice daily (approximately 0730 h and 1700 h) using a Model 330 Dissolved Oxygen Analyzer (Royce Instrumentation Corporation, New Orleans, LA). In anticipation of the presence of juvenile crawfish, diesel fuel was applied to the pond on 19 May 1988 at a rate of 46.7 l/ha to control predaceous insects.

#### Feed

At SFARU, all crawfish were fed a pelleted feed (Table 2) provided by Dupont Corporation and manufactured by Peoples Moss Gin (PMG), Palmetto, Louisiana. The feed was manually distributed to all ponds at a rate of 11 kg/ha every other day from 17 June 1987 to 27 April 1988 at which time the distribution rate in six ponds (A14, A15, A16, B14, B15, B16) was increased to 17 kg/ha every other day. The increase was in response to population estimates derived from sampling results. On 14 June 1988 the feeding rate was adjusted to 17 kg/ha every other day for the two remaining ponds (B12, B13). This feeding rate was maintained thereafter, until 31 October 1988. During the second growing and harvest season (1 November 1988–31 August 1989) feed was distributed at a rate of 17 kg/ha, every other day, except when the ponds were frozen over. Feed was distributed to all ponds between 1300 and 1500 h on scheduled feeding days. At DELTA, this feed was manually distributed at a rate of 17 kg/ha on Thursday and Saturday of every week from 20 May 1988 until the conclusion of the study.

TABLE 2.

Percent composition of experimental feed for pond aquaculture of crayfish.

Ingredient	Percent composition
Menhaden Meal	22
Rice Bran	15
Corn Meal	13
Soybean Meal	12
Wheat Midds	22
Blood Meal	8
Menhaden Oil	1
Aquabind	4
Vitamin and Mineral Premix	3

Mean of results of proximate analysis (percent as is weight) from two independent laboratories: moisture = 10.9, crude protein = 31.6, crude fat = 11.4, ash = 8.2, crude fiber = 4.9, nitrogen-free extract (as difference) = 33.0.

#### Trap Harvest

Traps were three funnel, open top, pyramid style, constructed with 1.9 cm plastic mesh (NAD Brokerage, St. Martinville, LA). These traps had an added feature of elongated necks to compensate for additional water depth not generally encountered in traditional crayfish ponds. The necks extended above the water surface and were fitted with PVC plastic retaining rings to prevent escape of crayfish over the top. Traps were set at a density equivalent to 75/ha.

AT SFARU, gizzard shad, *Dorosoma cepedianum*, was used as bait (0.23 kg/trap) from 1 April 1988 until 18 April 1988 when pond water temperatures were often below 17.0 C. Thereafter, Acadiana choice jumbo style bait was used. Bait was replaced either daily or every other day. In 1988, trap harvest was conducted and catch recorded from 1 April until 1 August. All trap-harvested ponds were harvested and rebaited once daily until the week ending on 28 May. Thereafter, harvest was conducted twice per week with traps set the previous day. Trapping effort was reduced in response to a decline in total weekly catch and to conserve labor. Trap harvesting was terminated on 1 August due to a consistently low catch. Trapping and the collection of population data continued but all crayfish were returned to the ponds except those females removed for ovary analysis. In 1989 trap harvest was conducted daily from 28 March to 17 July and thereafter, twice per week until 28 August. Acadiana choice jumbo style bait was used exclusively. At DELTA, crayfish were harvested three times each week (Thursday through Saturday) from 8 April 1988 until 1 August 1988. Traps were baited with one piece of Acadiana choice jumbo style crayfish bait. Harvest and sampling were suspended due to a substantial decrease in catch, low water level in the pond, and poor water quality conditions

(elevated water temperature and low dissolved oxygen levels) caused by a waterline break.

#### Seine Harvest

Seine harvest during 1988 was conducted at SFARU every other week from 1 April until 1 July with a 1.6 cm mesh seine (Memphis Net and Twine, Memphis, TN) having a weighted lead line. A total of six harvests per pond was conducted. Each harvest consisted of the amount of crayfish removed with one seine haul. Seine harvest was terminated during early July because of substantial declines in catch and to insure that sufficient egg-carrying females remained for sufficient fall recruitment. In 1989, seine harvest was conducted weekly from the first week of April to the first week of July.

#### Yield

Production data were collected from a total of 7 and 6 ponds for the 1988 and 1989 harvest seasons, respectively. Total harvested yield for each pond was calculated from the sum of all harvest efforts whether daily (trap), weekly (seine) or biweekly (seine). Feed conversion ratios, calculated as the ratio of the weight (as is) of feed to biomass harvested (wet weight) were determined for each production pond based upon the amount of feed provided from 17 June 1987 to 31 August 1988 (year 1) and from 1 September 1988 to 31 August 1989 (year 2).

#### Tail Meat Yield

Every other week from 18 April until 11 July 1988, a sample of 50 harvested crayfish representing several ponds was collected for analysis of tail meat yield. Crayfish were boiled for 10 min in unseasoned water and then stored overnight at 3.3°C. Thereafter, each crayfish was identified according to species, sex, and male reproductive state. Total length (TL) and whole cooked animal weight were also determined. The tail meat (abdominal muscle) was then removed and its weight, defined as the peeled (shelled) tail minus any adhering intestine or hepatopancreas, was determined. The percentage of whole animal body weight that was tail meat was calculated.

#### Texture

Samples of 6 crayfish harvested from each of 4 different ponds at SFARU (total = 24) and from the one pond at DELTA were taken once every 2 weeks during the period May 1 through July 15, 1988. No sample for DELTA was available for July. For each month the two monthly samples from each pond were combined for a total of 12 individuals/pond/month. Sixteen crayfish from each month's collection (four from each pond) were subjected to a penetration test and the remaining 32 (8 from each pond)

were subjected to a stress-relaxation test. The DELTA sample consisted of 12 animals, 4 and 8 being submitted to the penetration and stress relaxation tests, respectively. Comparisons were made between crawfish collected from SFARU and DELTA.

Texture measurements were performed with an Instron Model 1011 University Testing Machine (Instron Corp., Canton, MA). Test values recorded were mean values of duplicate samples. The first part of the texture test, the penetration test (Bourne, 1982), determined the peak penetration force in grams force ( $g_p$ ) necessary for a 2 mm diameter probe to pierce through the shell of a crawfish. Peak penetration force was recorded as the highest point on the curve. Instron crosshead speed was 20 mm/min, with the recorder speed proportional to the crosshead speed.

The second test, a stress relaxation test was similar to the test conducted by Weinburg and Angel (1985). In this test a peeled raw crawfish was placed on a solid support surface and an anvil compressed the sample to a 50% deformation level (Abide, 1988). The force required to compress the sample was recorded as stress force ( $g_p$ ). As the sample was held at this position, the time (seconds) required for this force to return to a level of 65% of its original position was recorded as the relaxation time ( $T_{65}$ ). Instron crosshead speed was 20 mm/min and recorder speed was 50 mm/min.

#### Statistical Analyses

One-way analysis of variance (ANOVA) was performed (SAS Institute, 1982) to identify if significant differences relative to tail meat yield existed during the harvest season at each site. All values expressed as a percent were arcsine transformed prior to analysis. If significance was indicated, a Least Significant Difference (LSD) multiple comparison test was used to identify significant pairwise comparisons. Possible differences in shell hardness or relaxation time related to the two geographical locations or time of year of harvest were investigated through a two-way analysis of variance using a  $2 \times 2$  factorial design. Differences were considered significant at the 0.05 level.

## RESULTS

#### Daily pH and Harvest—SFARU

No more than 10% of the daily pH levels recorded for all production ponds during the 1988 (Sept. 1, 1987–Aug. 31, 1989) and 1989 (Sept. 1, 1988–Aug. 31, 1989) seasons exceeded 9.5. These high pH readings were neither confined to an particular month nor occurred for any extended period of time during periods of recruitment, growth, and harvest. No relationship between the magnitude and incidence of high pH and yield for the harvest seasons of 1988 and 1989 was apparent.

For the 1988 harvest, the mean cumulative yield of all trap harvested ponds (926 kg/ha,  $n = 5$ ) exceeded that of seine-harvested ponds (525 kg/ha,  $n = 2$ ) (Table 3). Trap-harvested ponds stocked at 56 kg/ha ( $n = 3$ ) had a greater mean cumulative yield than seine-harvested ponds ( $n = 2$ ) stocked at the same density. The greatest production achieved in 1988 was 1,365 kg/ha. Feed conversion ratios ranged from 1.45 to 3.84 ( $\bar{x} \pm SE = 2.70 \pm 0.38$ ). For the 1989 harvest yields associated with a total of 6 ponds notably increased and ranged from 1122 to 2942 kg/ha. Conversion ratios ranged from 0.81 to 2.41 ( $\bar{x} \pm SE = 1.48 \pm 0.24$ ). Yields from trap harvested ponds averaged approximately  $1.9 \times$  those of seine harvested ponds for both harvest years.

During the period 1 April through 1 August 1988, total yield from daily trap harvest increased dramatically from the middle of April until the end of May and then leveled off (Fig. 1). After the third week of April, ponds stocked at 56 kg/ha consistently had a greater mean cumulative yield than those stocked at 84 kg/ha.

During 1988 the peak of the harvest season occurred during April and May (Fig. 2). For those ponds stocked at 56 kg/ha the mean monthly yield from trap harvest exceeded that from seine harvest except for May. The 1989 harvest showed a similar trend. In each of the ponds harvested in 1989, 75 to 90% of the total harvest occurred during the three month period of April, May, and June. At biweekly intervals throughout the 1988 study, the cumulative mean yield of trap harvest ponds exceeded that of seine harvest ponds with the lower stocking density (56 kg/ha) (Fig. 3). However, when a comparison on a single day basis could be made, the total yield of crawfish from two seine harvested ponds was between  $2-8 \times$  the combined yield from all six trap-harvested ponds (Fig. 4). In addition, the results suggest that a yield equivalent to that derived from one 20-min seine harvest in one pond would require the daily harvest of 72 traps located within a total of 24 different ponds. A similar relationship was also observed in 1989.

#### Harvest—DELTA Pond

The total harvest of 316 kg/ha was less than the total harvest from any of the ponds at SFARU. However, the number of trap days per week at DELTA was only 43% that for ponds at SFARU. A comparison of the proportional amounts of total yield according to month for the two study sites is provided in Figure 5.

#### Tail Meat Yield

The mean tail meat yields of *P. clarkii* collected from SFARU ponds on 18 April and 2 May 1988 were 23.3% and 21.8%, respectively, and were significantly greater than those for all other sample dates (Table 4). For the remainder of the sampling period, the yield ranged from 18.0

TABLE 3.

Total yield (kg/ha) from individual ponds harvested by either trap or seine during 1988 and 1989 at SFARU.

Harvest Procedure	Year	Pond <sup>1</sup>						$\bar{x}$
		A14	A15	A16	B13	B14	B15	
Seine	1988	558					491	525
	1989		1452				1122	1287
Trap	1988		1365	992	1137	510		926
	1989			1790	2289	2942		2406

<sup>1</sup> all ponds originally stocked at 56 kg/ha, except A16 and B14 (84 kg/ha)

to 19.9%. Yields for the 28 May and 13 June sampling dates were significantly greater than those of the 16 May and the final two sampling dates (27 June and 11 July). The reduction corresponded to an increase in Form I males which had significantly lower yields than Form II individuals and to an overall increase in size as reported by Niquette and D'Abramo (1991). Tail meat yield of females exceeded that of males throughout the sampling period at both sites. Form II (sexually immature) males had tail meat yields that were essentially equivalent to those of females. Crawfish fed strictly an artificial pelleted feed had an equivalent and often greater yield of tail meat when com-

pared with crawfish that exclusively consumed forage in various stages of decomposition.

#### Texture

Shell hardness, measured by penetration force and compression force, of crawfish from the 2 study sites was not significantly different. However, shell hardness of crawfish sampled in July at SFARU was significantly greater than that of individuals taken during the months of May and June. The mean relaxation time (6.7 sec) for the tail muscle of crawfish from the SFARU ponds significantly exceeded

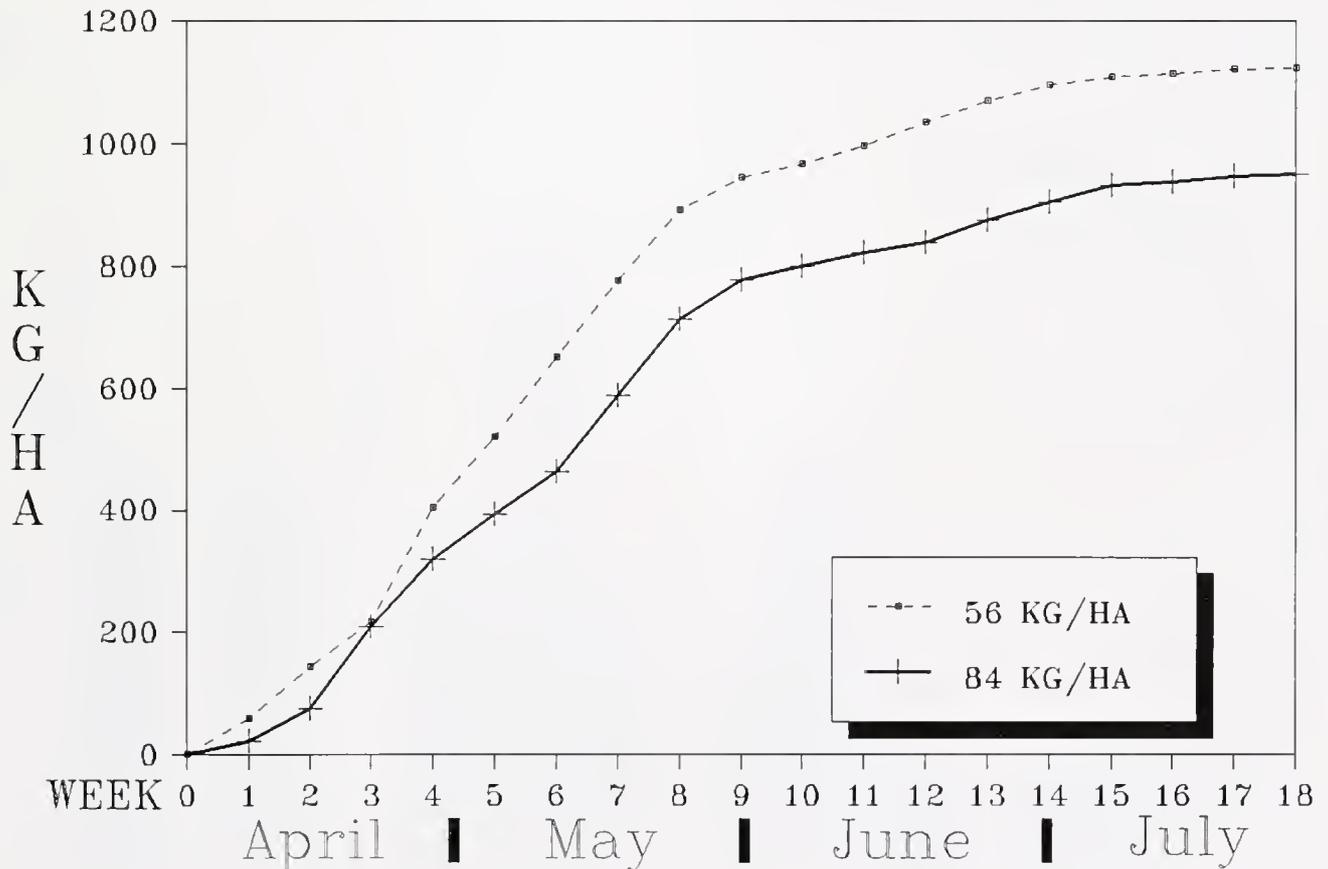


Figure 1. Mean weekly cumulative yield of crawfish from ponds stocked by 56 kg/ha (n = 3) or 84 kg/ha (n = 2) and harvested by trap at SFARU.

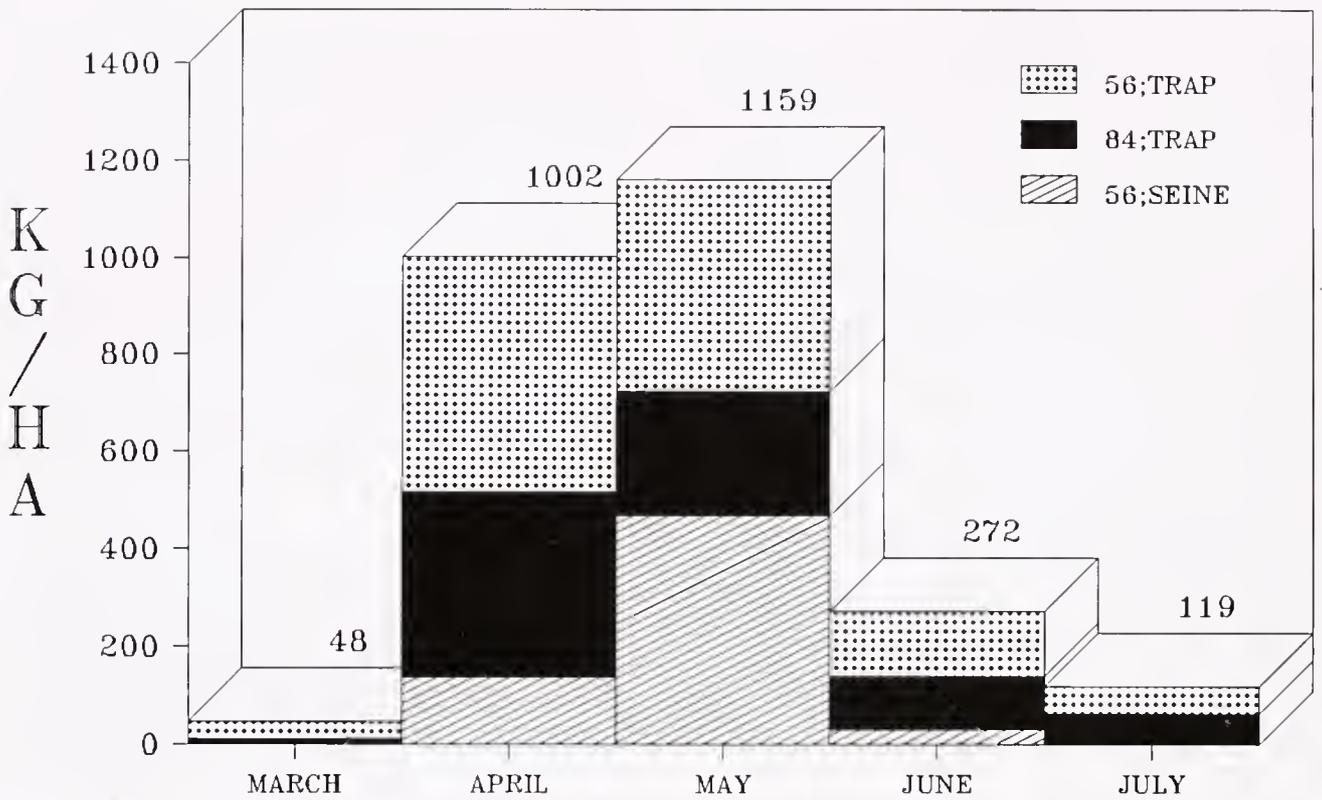


Figure 2. Mean monthly yield during 1988 at SFARU from two seine harvested ponds stocked at 56 kg/ha and each of two groups of three trap harvested ponds stocked at 56 kg/ha and 84 kg/ha, respectively.

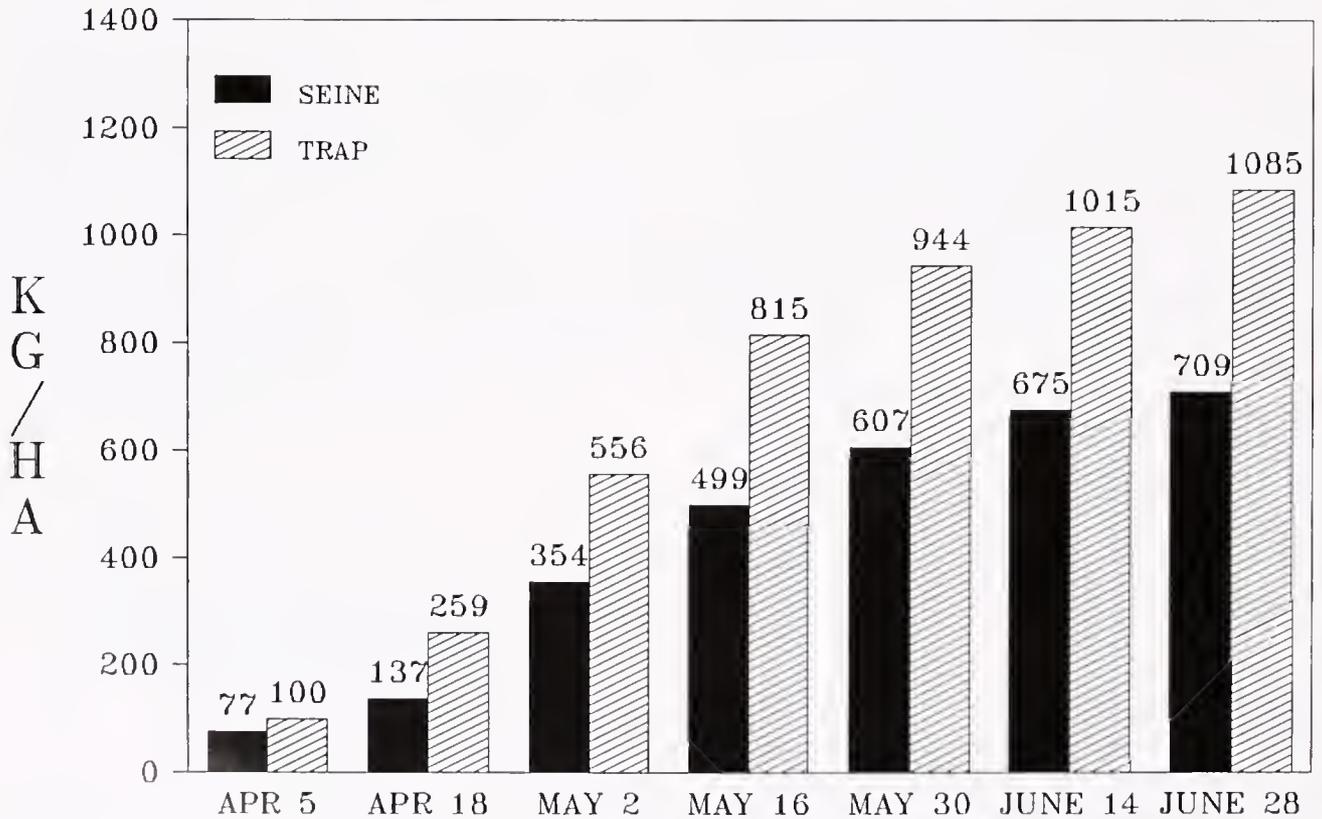


Figure 3. Cumulative mean yield expressed on an every other week basis from ponds stocked at 56 kg/ha and harvested either by trap or seine at SFARU.

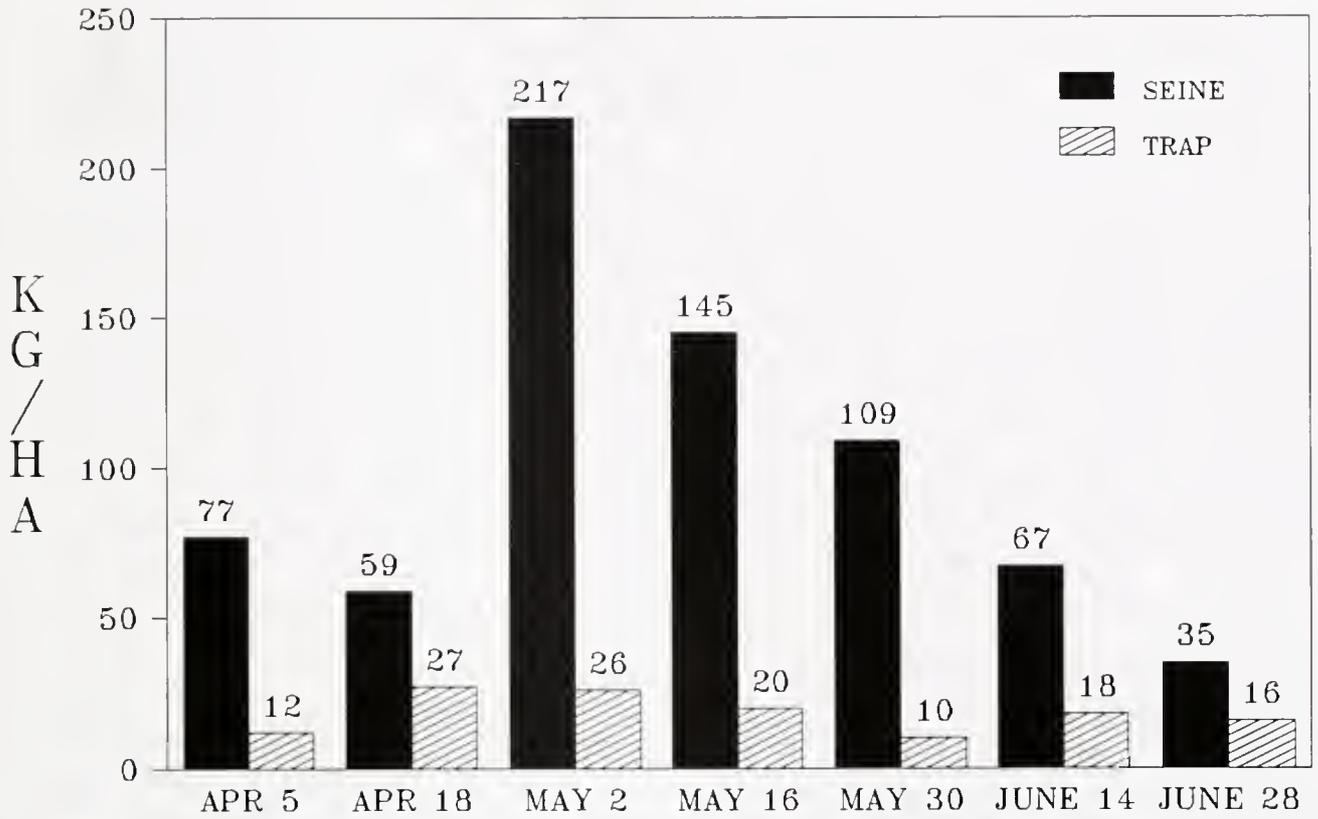


Figure 4. Total yield from two seine harvested ponds and six trap harvested ponds at SFARU stocked at 56 kg/ha on dates when comparison was possible.

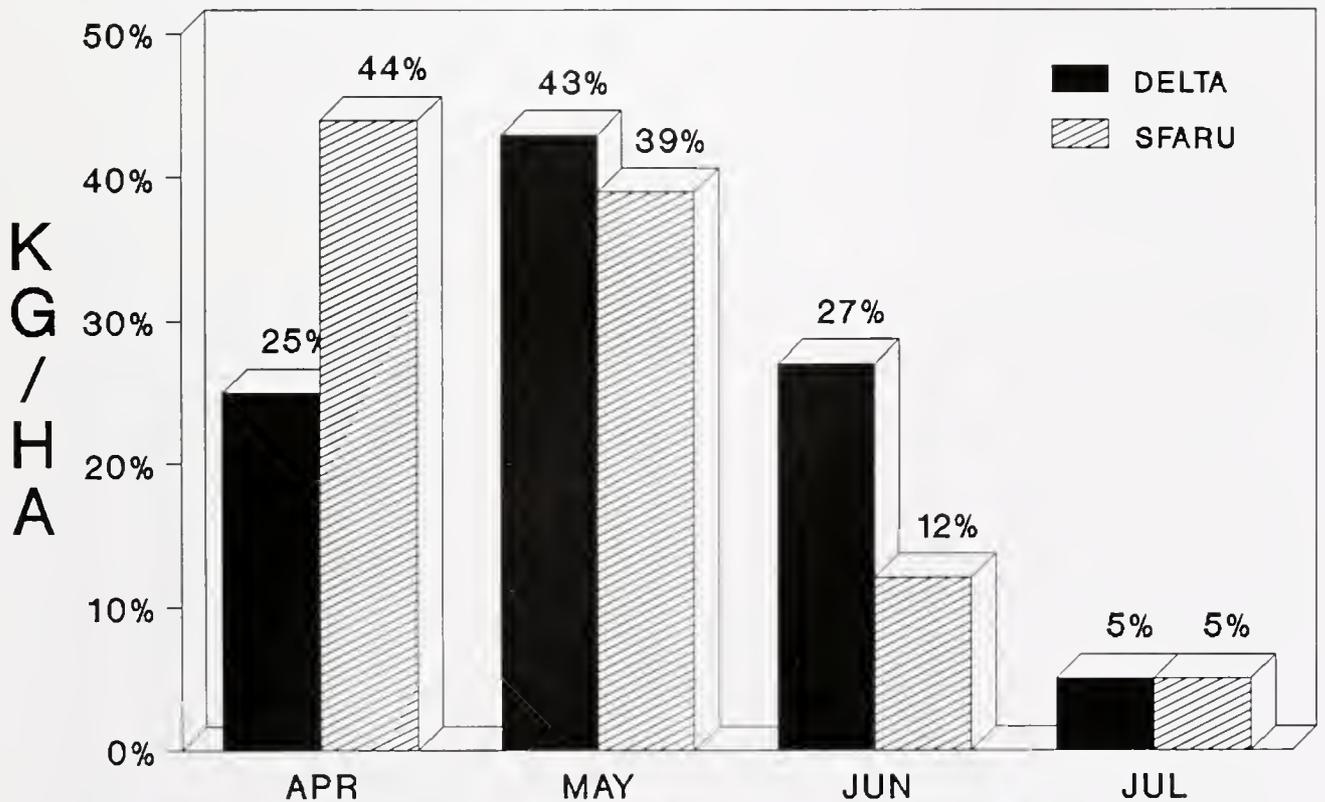


Figure 5. Proportion of total 1988 harvest for each month at the two study sites.

TABLE 4.

Mean percent tail meat yield by species, sex and maturity for biweekly samples collected at SFARU and the Delta site. (n) = number of observations per sample of 50 individuals.

	<i>P. clarkii</i>									
	Mean %		Mean % Male		Mean % Female		Mean % Form I		Mean % Form II	
	SFARU	Delta	SFARU	Delta	SFARU	Delta	SFARU	Delta	SFARU	Delta
18 April 88	23.3 <sup>a</sup> (44)	22.9 (21)	22.5 (20)	21.6 (17)	24.0 (24)	28.2 (4)	17.7 (2)	16.3 (7)	23.0 (18)	25.3 <sup>b</sup> (10)
2 May 88	21.8 <sup>a</sup> (47)	17.5 (37)	21.7 (31)	15.7 (24)	22.1 (16)	20.9 (13)	19.4 (11)	15.3 (23)	23.0 (20)	23.9 (1)
16 May 88	18.0 <sup>b</sup> (49)	17.8 (38)	16.9 (37)	15.9 (21)	21.3 (12)	20.2 (17)	16.9 (37)	15.9 (21)	—	—
28 May 88	19.9 <sup>c</sup> (49)	15.4 (44)	17.6 (23)	14.6 (29)	21.9 (26)	17.0 (15)	17.6 (23)	14.6 (29)	—	—
13 June 88	19.6 <sup>c</sup> (50)	19.4 (34)	18.5 (35)	18.1 (15)	22.3 (15)	20.4 (19)	18.2 (34)	18.1 (15)	27.8 (1)	—
27 June 88	18.0 <sup>b</sup> (48)	16.9 (34)	16.5 (31)	15.0 (18)	20.7 (17)	19.0 (16)	16.5 (31)	15.0 (18)	—	—
11 July 88	18.0 <sup>b</sup> (47)	20.1 (35)	16.9 (33)	17.5 (8)	20.7 (14)	20.9 (27)	16.9 (33)	17.5 (8)	—	—

<sup>a,b,c</sup> Means that do not share the same letter within a column are statistically significant.

that of crawfish from the DELTA pond (1.9 seconds). Crawfish from the DELTA site thus had tail meat that was less elastic than that of crawfish from the SFARU ponds. This site-specific difference in texture may be related to differences in the principal sources of food.

#### DISCUSSION

Exclusive feeding of formulated feeds and seine harvest have been demonstrated to be potentially viable management alternatives. The seven ponds that were judged to be satisfactory for evaluation in this study (i.e., having a reproducing population), had yields in 1988 that were comparable to those achieved in commercial ponds (Huner 1987). Yields from the 1989 harvest were generally greater than those normally encountered in the commercial U.S. crawfish aquaculture industry. The second year increase in yields follows the general observation of established ponds outperforming newly-started ponds (Huner and Barr 1984).

Increasing stocking rate from the standard 56 kg/ha to a rate of 84 kg/ha in 1987 did not achieve an increase in production during the following year. No relationship between mean harvest size and stocking rate was readily apparent. Variability of environmental factors may have exerted a greater effect upon production than the increase in stocking rate. Periodically, poor water quality, predation, crawfish migration, climate and other unknown factors can significantly influence production in individual ponds.

Successful daily trap harvest appears to be unreliable and dependent upon several environmental conditions. Seining as an active rather than passive harvest technique, removes a certain amount of uncertainty. As a result, daily catch per unit effort can be considerably increased with the adoption of a seine harvest.

Seining is particularly advantageous for rapid batch harvest and represents a more reliable procedure for meeting market demand, particularly on a short term basis. Large amounts of fresh crawfish, including soft shells, can be supplied for impromptu or routine need. Seine harvest could be conducted on a weekly or daily basis and entire ponds or portions of ponds could be harvested according to a rotational schedule. Because recruitment occurs in pulses, a planned seine harvest program would definitely increase production by selective removal of larger individuals. Such a procedure would serve to reduce resource competition and minimize the growth retarding effect of large crawfish on smaller individuals as described by Lutz and Wolters (1986).

More research is required to assess seines of different design and mesh size so that control of the size of crawfish harvested can be achieved. Nonetheless, annual yield realized by seining was consistently less than that achieved with traps. Performing seine harvests at dusk or in the darkness may prove to increase yield. Feeding just prior (15–20 min) to a scheduled seine harvest may also increase the catch. Effective seining can only be performed when the pond bottom is smooth and free of vegetation.

Results of the tail meat yield determinations were in agreement with a previous study that reported yields of females exceeding males of *P. clarkii* (Huner 1988). Analytical examination of shell hardness confirmed the previously documented (Huner and Lindquist 1985) belief that shell hardness does increase during the period from early spring to late summer. The hardness is probably associated with a particular physiological state or an environmental condition. Additional testing is required to determine whether the site-specific difference in tissue elasticity iden-

tified in this study can actually be discerned by consumers and thus become an important component of product quality.

The management approach used in this study was not successful in extending the growing and harvesting season. Huner et al. (1983) observed a decline in crawfish numbers through the summer in ponds that remained flooded in the summer. Our sampling revealed no summer recruitment (Niquette and D'Abramo 1991). Summer recruitment of *P. clarkii* is known to occur within roadside ditches (Huner 1975) and young-of-the-year released in August–September can mature by November–December (Avault et al. 1975, Huner and Avault 1976). Huner (1978) emphasized the importance of successful multiple recruitment in order to achieve maximum production. Extended, or off-season, pond production may be achieved through the spring recruitment of crawfish or supplemental stocking of juveniles in late spring or early summer derived from a hatchery operation (Trimble and Gaude 1988). Using this management approach 2 and possibly 3 crops per year might be realized. Recently, a delay in the normal management routine for pond culture of crawfish succeeded in extending the length of the harvest season (Eversole 1990). Other past research efforts devoted to "off-season" production have demonstrated biological feasibility but the prospects of economic success were reported as being unattractive (Romaine and de la Bretonne 1987). Recent efforts have demonstrated that summer crops of 560 to 1678 kg/ha may be achieved (Huner 1987, Culley 1987, Romaine and de la Bretonne 1987). Year-round supply of crawfish could become an important factor in the successful development and expansion of domestic and foreign markets.

Seine harvest and the provision of formulated feed will introduce needed control in the management of crawfish production ponds. Another logical step toward achieving reliable production relative to a size is the stocking of juveniles combined with an effective seine harvest schedule. This approach may be the most effective toward increasing revenue rather than merely increasing production by increasing the stocking density of broodstock as suggested by Chien and Avault (1980).

This study has provided fundamental information that is critical to achieving progress toward the intensification of commercial crawfish culture. Additionally, new avenues of research that include controlled stocking density and batch culture, use of a formulated feed, and alternative harvesting methods have been identified. Results indicate that goals of an extended growing season, greater yields, and a quality crop do have the potential of being realized if some or all of these management practices are successfully introduced.

#### ACKNOWLEDGMENTS

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## POPULATION DYNAMICS OF RED SWAMP CRAWFISH, *PROCAMBARUS CLARKII* (GIRARD, 1852) AND WHITE RIVER CRAWFISH, *P. ACUTUS ACUTUS* (GIRARD, 1852), CULTURED IN EARTHEN PONDS<sup>1</sup>

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**ABSTRACT** An evaluation of population characteristics associated with the culture of crawfish *Procambarus clarkii* and *P. acutus acutus* in experimental earthen ponds and a commercial pond was conducted. The experimental ponds were not drained, permitting assessment throughout the calendar year. Crawfish in these ponds were fed a formulated feed. The commercial pond was managed according to traditional practices (draining, planting of forage, filling). From September 1987 through August 1989 groups of crawfish were either permanently removed (harvested) from or returned to experimental production ponds. Crawfish  $\geq 75$  mm total length were harvested either by trap or seine. Species composition, sex, reproductive state, and juvenile presence were monitored.

Seine harvest provided a different and presumably more accurate assessment of the percentages of females and form I and form II males, and mean length of individuals from pond populations. In April, non-reproductive (Form II) males predominated while reproductively mature (Form I) males increased proportionately in May and thereafter. As the ovarian development of females collectively advanced their presence in traps declined. A gonadosomatic index (GSI) was developed for females and found to be an excellent indicator of the reproductive capacity and time of egg-laying. The largest pulse of juveniles occurred approximately one month subsequent to the peak in GSI. Juveniles were not part of the pond population from June through August.

**KEY WORDS:** crawfish, *Procambarus clarkii*, *P. acutus acutus*, population dynamics

### INTRODUCTION

Existing practices for the culture of red swamp crawfish *Procambarus clarkii* and white river crawfish *P. acutus acutus* in ponds as described by Avault and Huner (1985) are primitive, unreliable, and not conducive to achieving either maximum production or control of the size or quality of the product. A major problem associated with continued expansion of the industry has been lack of control of yield and population density (Romaine et al. 1978). No universally accepted method for determining population densities has been developed and information concerning population dynamics in ponds where crawfish are cultured is not well documented.

To maximize revenue from commercial pond production, departure from traditional practices in favor of more controlled culture is needed. Implementation of highly controlled culture, however, will require a thorough knowledge of population dynamics to maintain optimum conditions for growth, survival, and reproduction. This study was conducted to derive information concerning the dynamics of pond populations of crawfish throughout an entire calendar year and to compare assessment procedures.

### METHODS

#### Collection of Population Data

A description of pond sites, SFARU and DELTA, as well as pond management practices, and experimental design has been reported by D'Abramo and Niquette (1991). At SFARU, population data were collected once weekly from each trap-harvest pond for 33 weeks (last week of March through the last week of November, 1988). Individuals were collected by seine every other week from the second week of April through the last week in November. Seine collection of individuals was not successful during September and October. All crawfish collected from each pond on the sample day were included in the sample. However, if more than 100 individuals were collected from a pond a random sample of 75 was used to record population data. At DELTA, population data were collected 3 days/week for 17 weeks (8 April 1988–28 July 1988) and included either all those collected or a random sample of 100 if the total catch exceeded 150 individuals.

Each crawfish was externally identified according to species, sex, and reproductive state (males only), and total length (TL) were recorded. Form I (mature) males were identified by the presence of cornified gonadopods and distinct hooks on the ischial segment of their third and fourth walking legs. TL (mm) was measured from the distal tip of the rostrum to the distal point of the telson. Wet weight of individual crawfish was determined to the nearest 0.1 g.

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At both sites, a weekly sample of 10–15 *P. clarkii* and *P. acutus acutus* females >100 mm TL were selected during the period 18 April–26 July 1988 from a combined daily collection from all ponds. Additional samples were taken during the months of August and November at the SFARU site. Females of this size were selected to increase the likelihood of encountering ovarian development and were then stored at approximately 3.0°C for 24 h. Thereafter, weight and TL were recorded and the ovaries were removed and weighed. Color of the ovary for each female crawfish was recorded as either white (WH), yellow (YW), tan (TN), brown (BR), or black (BL). Ovaries without eggs were classified undeveloped (UD). Percentage occurrences of ovarian colors were calculated for each weekly sample and mean monthly percentages were also calculated for each study site. A gonadosomatic index (GSI) defined as the ovary wet weight divided by the whole crawfish wet weight and multiplied by 100 was used to quantify the degree of ovarian maturation. A range of GSI values for each color was developed by plotting all the GSI values in each color group. Values shared by two color groups were assigned to the group having the lower range of values.

Juvenile crawfish ( $\leq 75$  mm TL and  $> 10$  mm TL) were sampled weekly (final week of March 1988 through the last week in January, 1989) using one three-funnel, open top, pyramid trap set for one day. The trap was constructed with 3.2 mm plastic mesh and a PVC plastic retaining ring. Another technique for sampling the juvenile population used a 35.5 cm by 28.0 cm dip net with 3.2 mm nylon mesh. Four 1 m sweeps were performed around the periphery of each pond (center of each side) once a week. All juveniles were counted, measured and then returned to their respective pond.

#### Statistical Analyses

One-way analysis of variance was used (SAS Institute 1982) to identify if significant differences over time existed relative to either the percentage of type 1 males or GSI. All values expressed as percentages were arc sine transformed prior to analysis to normalize distribution. If a significant difference was indicated, a LSD multiple comparison test was used to make pairwise comparisons. Differences were considered significant at the 0.05 level.

## RESULTS

#### Ovarian Maturation

Crawfish ovaries were initially observed as white and undeveloped. As development continued the size increased and color progressed through various shades of yellow, tan, brown, and eventually black (Fig. 1). According to the color designation, ovaries of females harvested from both sites were in at least three stages of development during the

mid-May through July period. Data collected at SFARU indicate two waves of ovarian maturity: one in July, and one during late winter as indicated from females obtained in November (0.155). The relative percentages of ovary colors from April–July suggest that ovarian development at SFARU was less rapid than that of crawfish from DELTA. Females with brown ovaries were observed as early as April at DELTA and May at SFARU. Black ovaries in females from the DELTA pond were first observed in July when the mean monthly water temperature was 29.1°C. Black ovaries at SFARU were not observed until August when the mean monthly water temperature had increased to 29.0°C. By then all ovaries of all sampled females were either brown or black.

A range of calculated GSI of female crawfish, in conjunction with the observed color of the ovaries, proved to serve as an excellent index of reproductive state. The range of values and corresponding ovarian color were 0–0.9 (white), 0.1–0.24 (yellow), 0.25–0.34 (tan), 0.35–0.69 (brown) and 0.70–2.0 (black). There was no apparent relationship between size (weight or length) and the degree of ovarian maturity.

Mean monthly GSI values at SFARU and DELTA along with the corresponding average water temperature are shown in Figure 2. At SFARU, the GSI value in August was significantly greater than that of July. Both July and August values were significantly greater than all other months (April, May, and June). At DELTA, the GSI value in July was significantly greater than that of April, May, and June. There was no significant difference for comparisons between June and April and between April and May. Although 11% of the females harvested from DELTA during July had black ovaries and no ovaries of females at SFARU were in this advanced state of development (Fig. 1), females at SFARU had a significantly higher mean GSI indicating either a larger number of eggs or larger size eggs, both suggestive of greater reproductive potential. No collections were made beyond July at DELTA thereby precluding a determination of whether the GSI had actually reached its peak. The final recorded GSI value of 0.548 at DELTA in July was less than the peak value of 1.941 at SFARU (recorded in August) suggesting that the maximum value had yet to be observed.

#### Population Parameters

*Juveniles.* Monthly percentage of *P. clarkii* collected by either dipnet or juvenile trap always exceeded 88%. *P. acutus acutus* was captured only in the early spring and late fall. The mean TL of juveniles caught with "juvenile" traps was consistently greater than that obtained with dip netting. Traps also caught more juveniles than the dip net (Fig. 3). By June, juveniles were no longer evident, suggesting that young of the year (y-o-y) had achieved a harvest TL of 75 mm. In September, juveniles began reap-

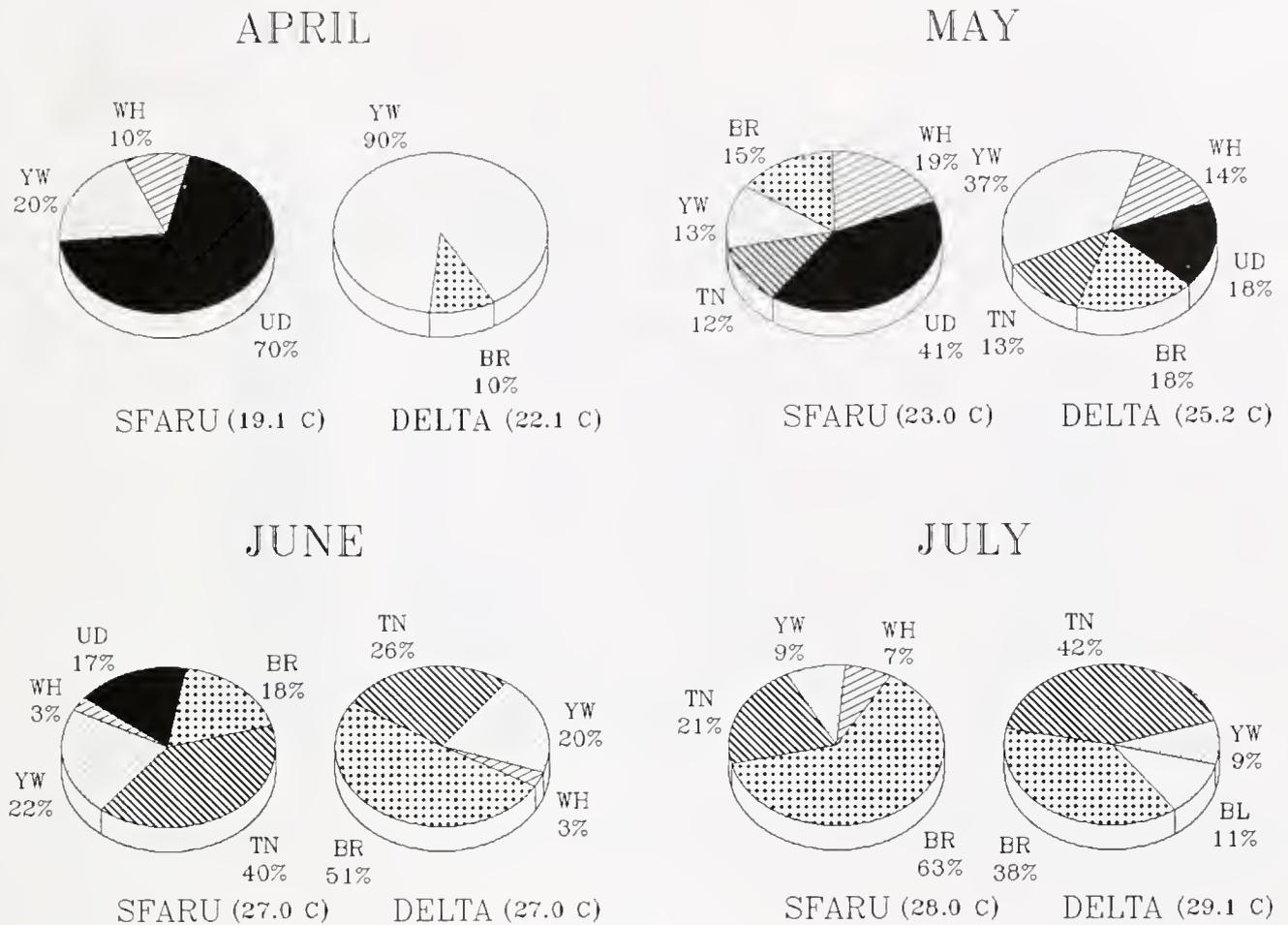


Figure 1. Monthly percentages of ovarian color and mean monthly pond water temperatures at SFARU and DELTA in 1988. ND = non-developed, WH = white, YW = yellow, TN = tan, BR = brown, BL = black.

pearing in the collections, approximately one month after egg laying (GSI peak in August). The number of juveniles in the pond populations had increased dramatically in November and remained comparatively high in December and January, indicating recruitment for the following year. The decline of young sampled in December and January suggested that the initial pulse of juveniles (September) had reached a harvest length.

**Male Maturity.** Monthly percentage of Form I males that were trapped peaked at 98 in July (Fig. 4). Percentage of Form I males from the seine-harvested population was 80% at this time and peaked at 89% during August. Form II males predominated in the April collections. In May, the proportion of Form I males substantially increased and by June exceeded 80% of the males collected. From April through August, a greater mean percentage of Form II males was collected from seine than from trap harvest. The percentage of Form I males harvested by seine was significantly greater in June, July, and August when compared to April. In July and August percentages of Form I males harvested by trap at SFARU were significantly greater than the

percentage of Form I males in April, May, and November. There were no significant differences in the percentage of Form I males in June, July, August, September, and October. The highest percentage of Form I males occurred in June at DELTA, approximately one month earlier than in the ponds at site SFARU. At both locations, the highest monthly GSI value was recorded one month subsequent to the highest monthly percentage of Form I males obtained from trap harvests.

**Male/Female Ratios.** Sample size was dependent upon the total number of crawfish harvested weekly from April–September and ranged from 38 to 1738 and from 342 to 1497 for SFARU and DELTA, respectively. The proportion of males caught at the DELTA site increased during the first three weeks of April. A decline began in late April and continued until June when the percentage stabilized at 50%. In contrast, the percentage of males trapped from ponds at SFARU increased from late March until the beginning of June, then essentially plateaued at 60% until August. During August, the percentage of males increased and then began a sharp decline in mid-September. At

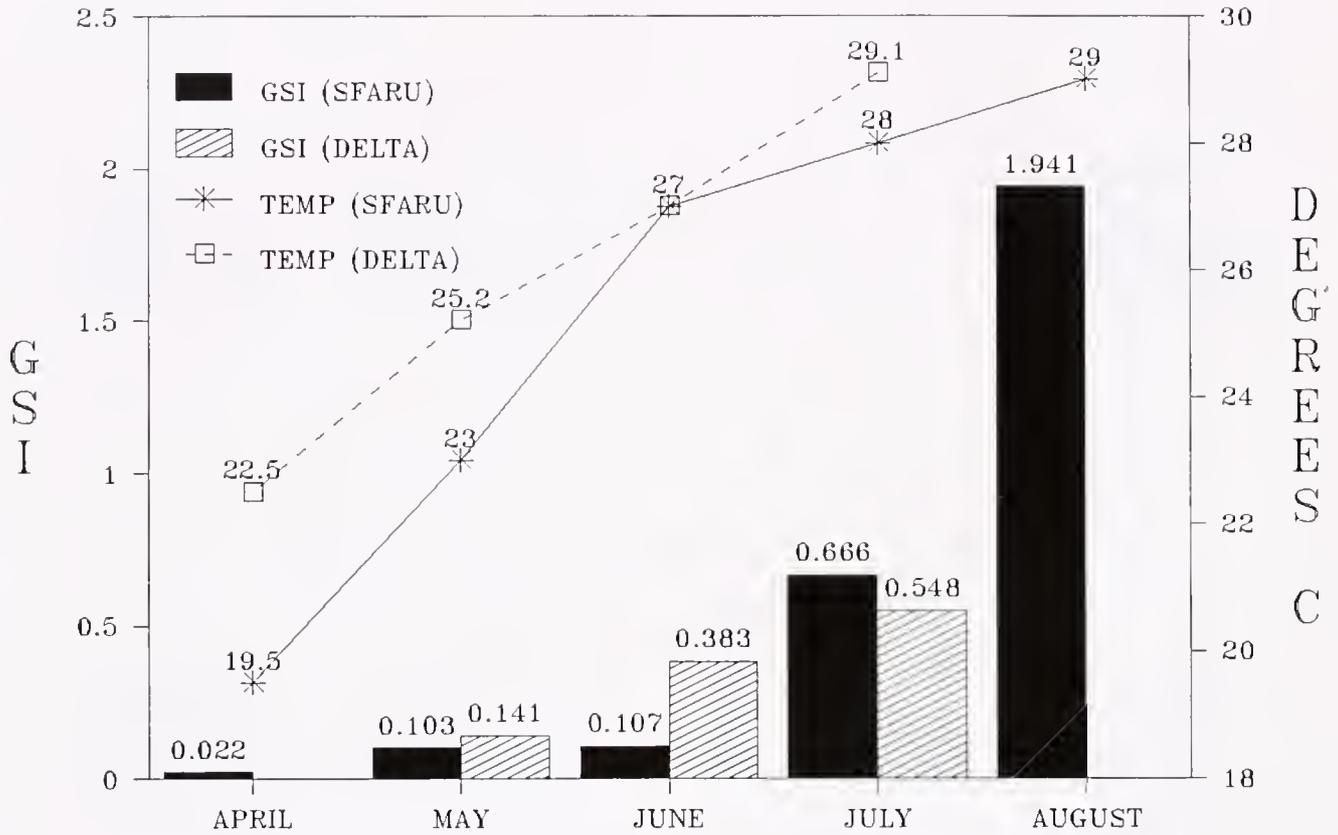


Figure 2. Mean monthly GSI and pond temperatures at SFARU and the DELTA sites during the 1988 harvest season.

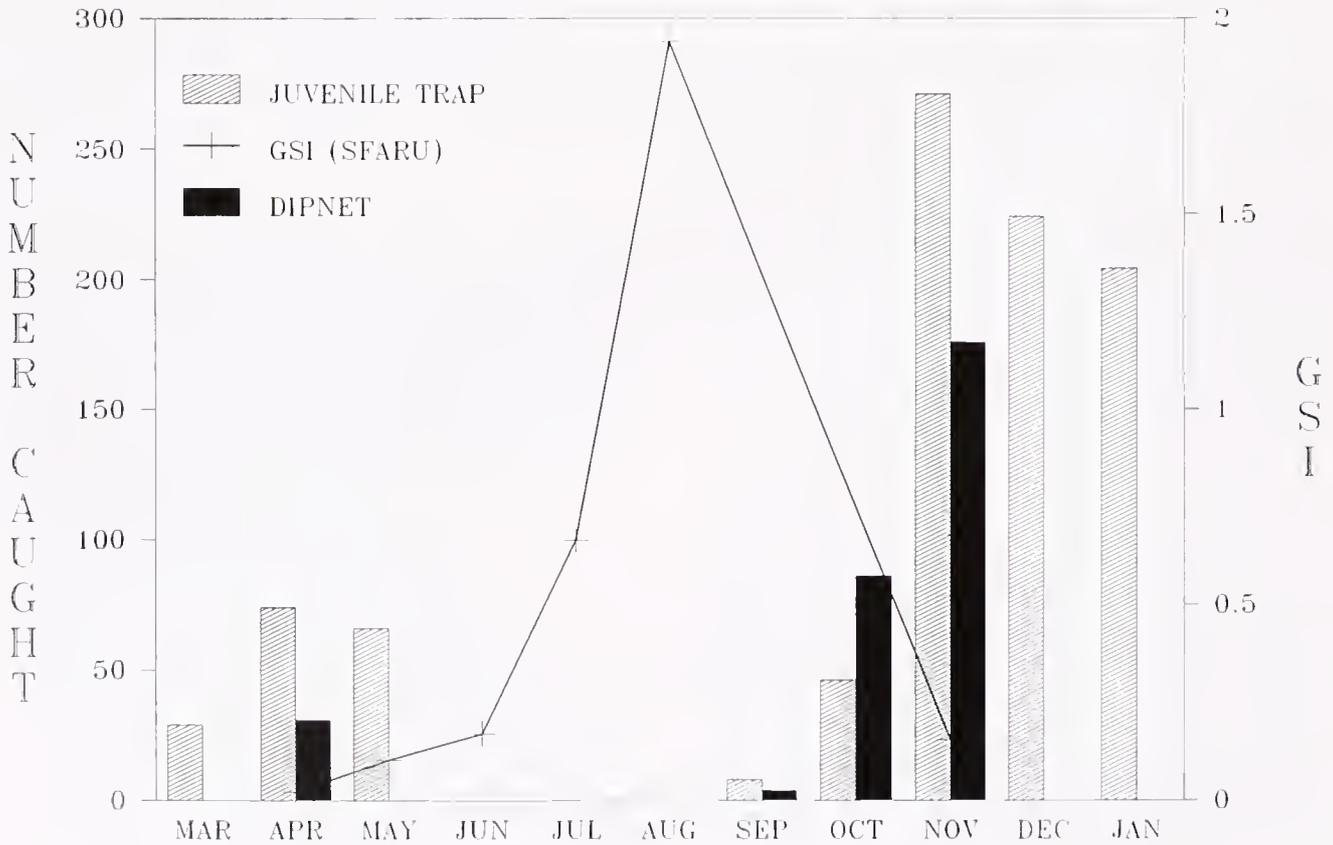


Figure 3. Total number of juveniles (<75 mm) collected monthly by either dipnet or trap and mean GSI of adult females collected from ponds at SFARU during 1988 and early 1989. No GSI data were collected for September and October.

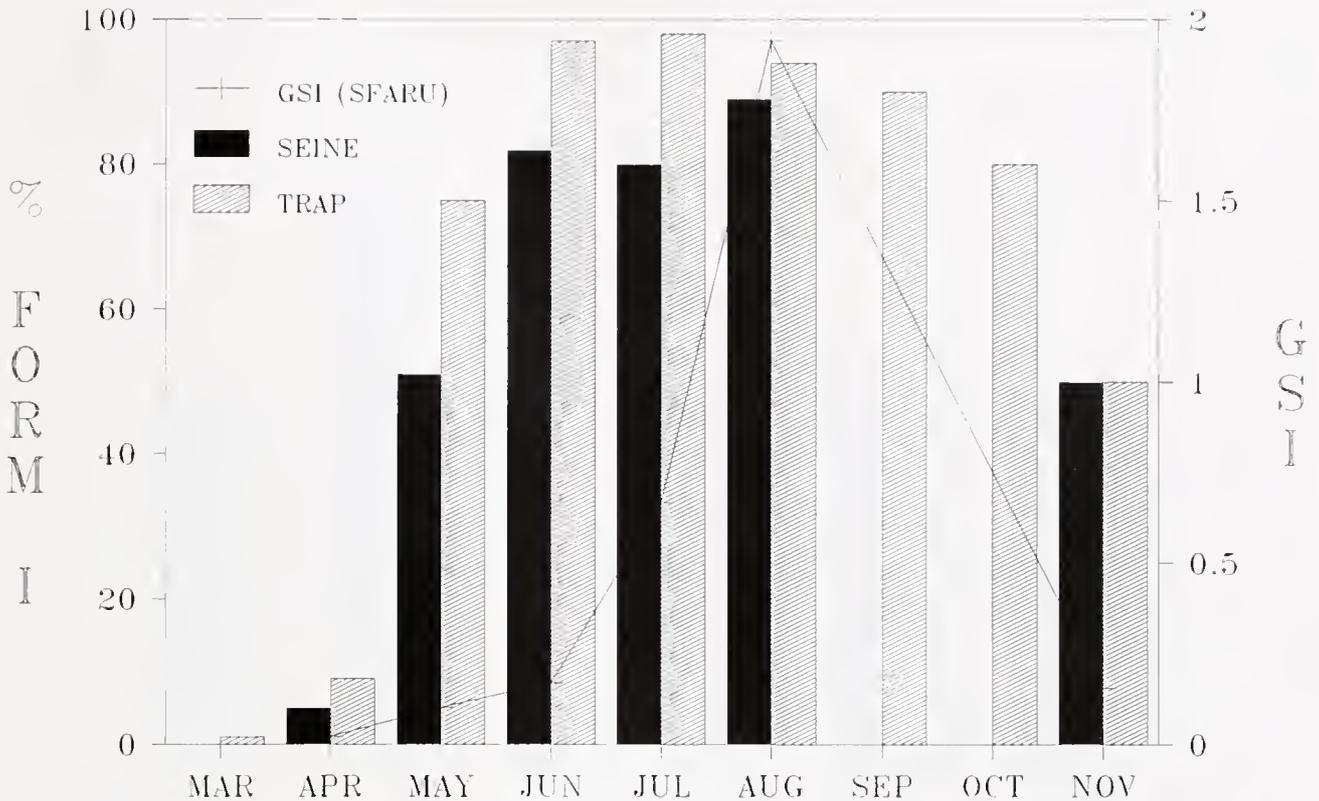


Figure 4. Monthly percentages of Form I males (mature) harvested by either trap or seine and mean GSI of adult females collected from ponds at SFARU during 1988. No individuals were collected by seine during September and October and no GSI data were collected for September and October.

SFARU, the overall percentage of females declined slightly from 49% in April to 42% in July with a low of 33% recorded in August. This distinct reduction in females in August corresponded with a peak in the GSI. At DELTA, the monthly percentage of females and the GSI value increased from 33% and 0.141 in May, to 54% and 0.548 in July.

Throughout the entire harvest period, more males than females were caught in traps and the ratio approached 1:1 during May, June, July and October for the seine-harvest ponds (Fig. 5). During August and September, the percentage of females collected by seining was approximately two times greater than that collected by trapping.

*Species Composition.* The relative proportion of harvested *P. clarkii* generally reflected that of the initial stocking population. *P. clarkii* dominated harvests from SFARU composing an average of 94.0, 98.6, 99.0 and 96.9% of the total number of individuals harvested during April ( $n = 2069$ ), May ( $n = 1179$ ), June ( $n = 603$ ) and July ( $n = 255$ ), respectively. Small intermittent increases in the percentage of *P. acutus acutus* were observed in weekly samples collected during August (26%,  $n = 43$ ), September (27%,  $n = 15$ ) and October (17%,  $n = 36$ ) but the overall percentage of *P. clarkii* caught during these months ranged from 90.2 to 96.7%. The presence of *P. acutus acutus* was attributed to an overall decrease in

sample size as well as decreases in water temperatures occurring at this time. At DELTA, the percentage of *P. clarkii* never exceeded 90% and *P. acutus acutus* comprised 25–75% of the harvest through mid-May. The original stocking population at DELTA had a greater proportion of *P. acutus acutus* than that of site SFARU. Trapping of *P. acutus acutus* was most effective during the early spring season.

*Change in Mean Length.* During April and May mean weekly total length (TL) of trapped crawfish ranged from 85 to 92 mm with monthly averages of  $87 \pm 0.21$  mm and  $88 \pm 0.35$  mm, respectively, at SFARU (Fig. 6). Seine harvested crawfish had mean TLs ranging from 88 to 97 mm during April and May. At DELTA mean TL for the months of April and May were  $92 \pm 0.34$  mm and  $100 \pm 0.28$  mm, respectively. No increase in length of crawfish harvested from site DELTA occurred from May through July with a substantial decrease in size seen during the last week of July. From May through the end of August monthly increases in mean TL of approximately 6–7 mm were observed in both trapped and seine harvested populations. Seine harvested crawfish were consistently larger than trap-harvested crawfish at SFARU during May, June, and July. Mean monthly TL peaked in July ( $104 \pm 0.28$  mm) and in September ( $104 \pm 1.11$  mm) for seine har-

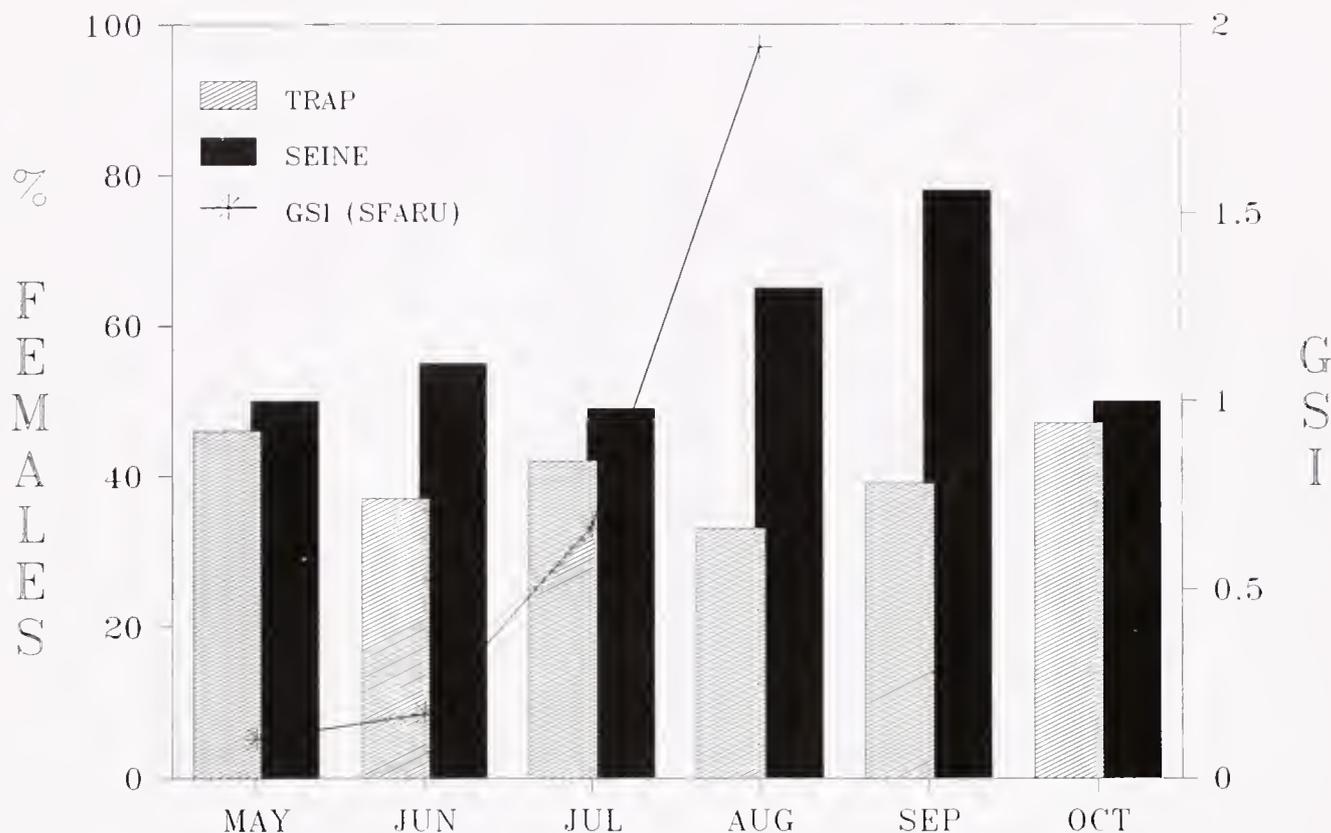


Figure 5. The percentages of females collected from ponds by either trap or seine during April through October and corresponding GSI values (April through August) at SFARU.

vested and trap harvested populations, respectively. A gradual and consistent increase in TL of crawfish harvested from ponds at SFARU throughout the study suggests that a balanced population structure existed. Growth rates could not be determined because a mixture of cohorts were being removed without replacement.

#### DISCUSSION

Although sampling at the DELTA site was not as frequent as that at the SFARU site, the population information derived provides for a comparison between the traditionally managed crawfish culture pond at the DELTA site with the SFARU site ponds that were under an unconventional management scheme. Nevertheless poor overall pond management at the DELTA site was probably the principal reason for low recruitment as indicated by the sampling. At this location the majority of crawfish caught in April probably were hold-over juveniles while those caught in May, June, and July were predominately hold-over adults because the mean TL barely changed. Poor recruitment was supported by the total lack of juveniles caught either by small mesh traps or dip net until November when only a few were found. Poor reproduction and juvenile survival would be conducive to a reduction in population levels and a low yield. Active sampling (seine) and passive sampling (trap)

provide different pictures of the dynamics of crawfish populations. It appears that seine sampling would provide a more accurate estimate of certain population parameters.

For example, seine sampling indicated that two times the number of females collected by trapping were actually present during and just prior to the egg laying period in August and September as determined by a maximum GSI value. The distinct absence of females in traps during this time confirmed previous speculation that females in an advanced reproductive state are less likely to enter traps. Apparently, females are more seclusive and may be building burrows during this time. These results are supported by previous observations with other species of crawfish. Bayrak (1985) found that the percentage of female *Astacus leptodactylus* caught with traps in a Turkish lake dropped markedly during the egg-laying period. After hatching, the ratio of females again represented almost 50% of the total catch. The disappearance was attributed to ovigerous females becoming secretive or avoiding traps prior to the hatching of young. Lake and Sokol (1986) found that a sex ratio of the Australian yabbie, *Cherax destructor*, was not significantly different from 1:1 in seine harvests whereas the ratio associated with trapping significantly favored males.

Traditionally managed crawfish ponds are drained

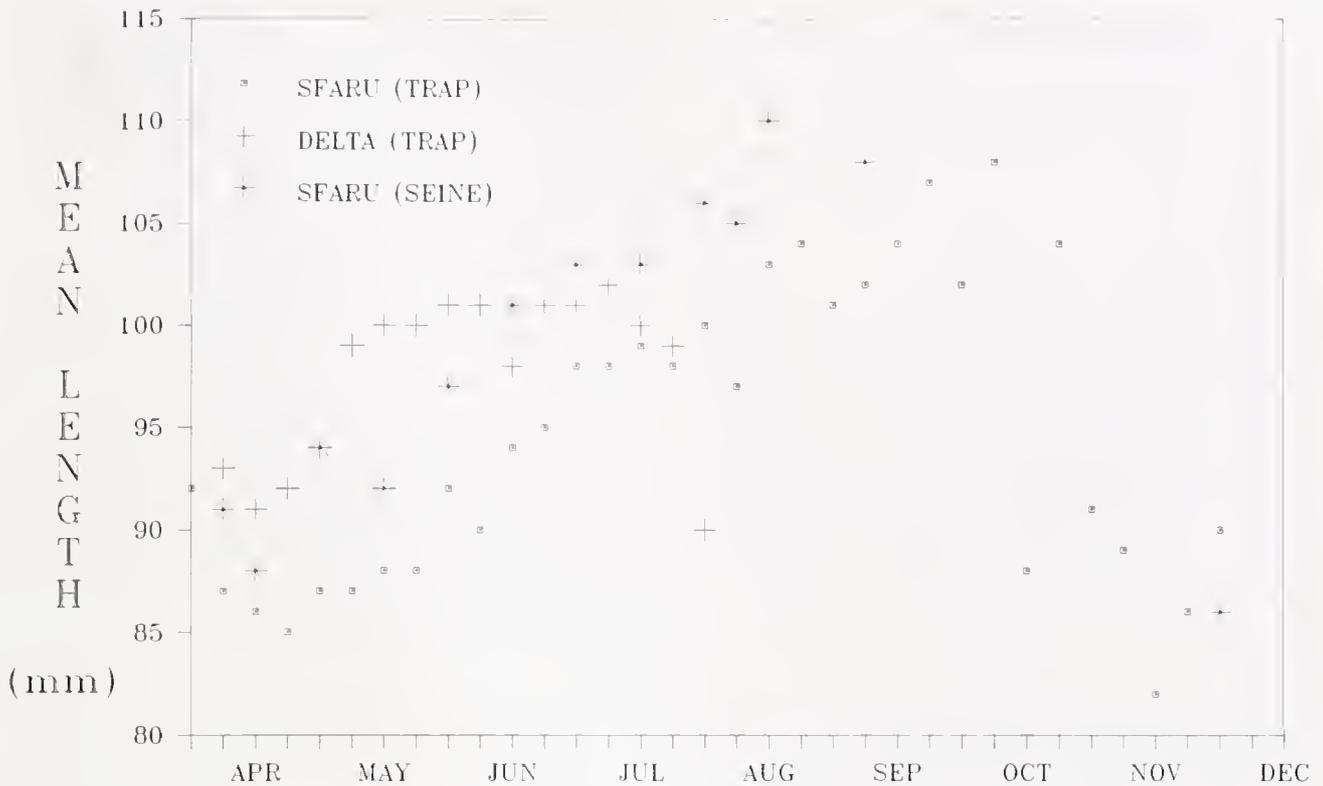


Figure 6. Mean weekly lengths (mm) of harvest size crawfish sampled by trap or seine from ponds at SFARU and the DELTA site.

during the later stages of ovary development. The non-draining of ponds at the SFARU site permitted the collection of data that identified the time of the onset of egg laying. Prior to this study, the degree of ovarian maturation was based exclusively on color. Use of color is very subjective and alone represents a qualitative estimate of the state of egg development. The development of a crawfish gonadosomatic index (GSI) proved to be a useful tool to quantify the state of ovarian maturation and therefore predict the time of egg laying. GSI serves to associate a certain range of numbers to one specific color of ovaries, thereby eliminating the high variability innately associated with subjective interpretation of color. Yet the potentially greatest, yet unestablished, utility of the GSI may be the description of the reproductive quality (capacity) of the female. GSI values that are found to be lower than that expected for a particular color would suggest that recruitment for the following year would be less than ideal. For example, a female determined to have a GSI of 2.0 may have greater reproductive potential than an equal size female with a GSI of 1.0 when both possess black ovaries. Sampling numerous females can therefore produce an index of the reproductive capacity of the entire population.

Females possessing brown ovaries during the spring were most likely left from the previous year and were carrying their second, third, or possibly their fourth set of eggs. The brown eggs carried by females in May, June, and

July in the SFARU ponds probably hatch in September, October, and early November. Those females that were removed in August and found to have brown or black ovaries were probably responsible for the juvenile recruitment which peaked in November.

Although the majority of harvested individuals at both study sites consisted of *P. clarkii*, the proportionately greater number of *P. acutus acutus* individuals harvested in early spring and late summer and fall supports the contention that this species is more active than *P. clarkii* at cooler water temperatures (Avault and Huner 1985). Harvest of both species may also be associated with the time of egg laying (Avault and Huner, 1985).

In this study the combined use of small mesh traps and dip nets effectively served to monitor the presence of juveniles, the approximate time of hatching for observed pulses of young crawfish, and the growth patterns of maturing young through the fall and winter months. Gabel (1988) stated that small mesh traps were the less accurate of the two sampling methods because very small crawfish and those individuals unattracted to bait are not included. Huner and Barr (1984) stated that as crawfish approach 50 mm in TL they become difficult to catch with a dip net. The mean TL of juveniles collected by trap in this study was essentially greater than or equivalent to that of juveniles collected by dip net. The poor results obtained with dip net versus trap sampling in March and May probably

reflects the avoidance of shallow water by juveniles during the day, especially when cover is lacking. In ponds where forage is not planted and volunteer aquatic vegetation is essentially absent, small mesh traps would appear to be most suitable for monitoring of juvenile populations, particularly if sampling is conducted during daylight hours. In the absence of cover young crawfish tend either to remain in deeper water or congregate in isolated patches of cover such as algae. Experience gained from this study suggests that both collection techniques should be used to obtain a representative sample. However, using small mesh traps and dip nets to obtain accurate estimates of population density of juveniles does not appear practical.

Estimations of mean growth rate were not possible in this study because proportions of certain length classes were regularly harvested from the population. Moreover, growth rates are probably density dependent, as demonstrated in juveniles of *Astacus astacus* (Ackefors et al. 1989), and based upon the management employed in this study, the number and total biomass of crawfish in a pond

at any particular time cannot be confidently estimated. An accurate determination of growth rates might be achieved from analysis of two consecutive seine harvests within a short period of time (7–10 days). Alternatively the controlled stocking of juveniles followed by routine monitoring of TL of individuals from samples taken during the growing season could be used.

The same population parameters were monitored in ponds at the SFARU site for an additional year (August 1988–August 1989), using the materials and methods already outlined. Relationships and trends depicted for 1988 pond populations were similarly observed the following year (November 1988 through November 1989). Collection of fundamental and accurate biological information is critical to achieving progress toward the intensification of commercial crawfish culture. The results of this investigation should assist in the development of new management practices that will be conducive to achieving future goals of an extended growing season, greater yields, and a product that meets the demand of the consumer.

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## OBSERVATIONS ON NOBLE CRAYFISH, *ASTACUS ASTACUS LINNAEUS*, (DECAPODA, ASTACIDAE), POPULATIONS IN CENTRAL FINLAND—MANAGEMENT IMPLICATIONS

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**ABSTRACT** Observations of well-established, non-expanding *Astacus astacus* populations in central Finland show that males and females mature at sizes of 7–8 cm total length at 4–6 years of age. Though mature males are sexually competent each year, most mature females spawn in alternate years. Mature males grow larger than mature females because they may molt twice during each summer while mature females molt only once. Mortality is high in both sexes above 9 cm with more males than females reaching the legal size limit of 10 cm. Most females spawn twice by the time they reach the 9–10 cm size range and disappear from the population. Therefore, the 10 cm size limit should be reconsidered.

**KEY WORDS:** freshwater crayfish, management, *Astacus astacus*

### INTRODUCTION

The noble crayfish, *Astacus astacus*, is the dominant macrobenthic invertebrate in many Finnish freshwater ecosystems (Huner and Lindqvist 1986–1988, Westman and Pursiainen 1982). Its presence in or absence from suitable littoral substrates are largely dictated by the virulent crayfish fungus plague, *Aphanomyces astaci*, which first appeared in the country in 1893 (Westman 1975). However, changes such as stream channelization can be locally important in eliminating habitat (Niemi 1977). Noble crayfish are much more common in smaller water bodies than larger interconnected lakes because these apparently provide refuges from continuous exposure to *A. astaci* (Huner and Lindqvist 1988). It should be noted, however, that no one has yet explained the mechanism(s) by which *A. astaci* persists despite the apparent total elimination of its only known host during plague episodes (Alderman and Polglase 1988).

The noble crayfish is an especially important fishery resource in Scandinavia (Westman et al. 1990). It is an important component of the traditional late summer crayfish parties held throughout the region. Scarcity and demand generate high prices. As a result, the fishery for this species is the most valuable freshwater fishery in the region. Demand is so great, in fact, that large volumes of cooked, frozen freshwater crayfishes are imported from around the world.

The commercial availability of *A. astacus* in Finland is affected by a minimum size limit of 10 cm total. This limit was established in the 1890s apparently on the basis of purely commercial considerations. This size limit can be

compared to and is higher than size limits of 9.0 cm and 9.5 cm total length, respectively, in neighboring Sweden and Norway (Westman et al. 1990).

Noble crayfish mature after 4–6 summers at sizes of 7–8 cm in Scandinavia (Svärdson 1949, Abrahamsson 1966, 1972, Westman and Pursiainen 1982, Huner and Lindqvist 1986, Skurdal and Qvenild 1986, Westman et al. 1986, Taugbøl et al. 1988, Lahti 1988, Pursiainen et al. 1988a, b, Westin and Gydemo 1988, Huner et al. 1990). Mating and spawning occur in early autumn. At Finnish latitudes, females brood young well into the following summer. Most brooding females molt well after males, immature females and non-reproductive, but mature, females. As a consequence, these females have insufficient time to accumulate energy reserves to both persist through the following winter and to regenerate ovaries and spawn before winter comes. Therefore, in Scandinavian countries at similar latitudes, reproductive activity of females commonly occurs in alternate years (Huner and Lindqvist 1988, Pursiainen et al. 1988b, Huner et al. 1990).

A review of *A. astacus* population structures and growth increment per molt based on modal length frequencies in Scandinavia (Svärdson 1949, Abrahamsson 1966, 1972, Westman and Pursiainen 1982, Huner and Lindqvist 1986, 1988, Skurdal and Qvenild 1986, Westman et al. 1986, Pursiainen et al. 1988a, b, Taugbøl et al. 1988, Huner et al. 1990) shows that mature female *A. astacus* of 7.5–8.0 cm will have spawned twice upon reaching 9.0–9.5 cm provided the fishing seasons begin in mid-late summer. We present here data summarizing our observations of several *A. astacus* populations in central Finland during the 1980s. These strongly indicate that mortality between 9 and 10 cm

is extensive in well-established, non expanding populations. This supports, then, the need to reconsider the current 10 cm size limit.

#### MATERIAL AND METHODS

We have collected crayfish with baited folding traps, 0.9 cm square mesh, or from commercial fishermen who used baited traps, 1.7 cm square mesh from summer 1987 to mid-summer 1990. Most of the crayfish were collected during July–September after the summer molt. Descriptive data on study sites including locations and limnological considerations are presented in Table 1. Sites ranged in size from South Valkealampi Pond, approximately 5 ha to Lake Suur-Lausjärvi, approximately 603 ha in size. All sites were ponds or lakes with the exception of River Kutujoki. Waters in the study sites have a wide range in conductivity ranging from about 30 ohms/square centimeter in the two large lakes to near 200 ohms/square centimeter in the smaller ponds near Kuopio. This was reflected in total alkalinities and total hardness levels. July and August temperatures in littoral habitats favored by crayfish have been in the 17–22 C range depending on the weather during a particular year. Late winter temperatures are 1–2 C. Oxygen levels have been near saturation in the littoral zone and water clarity is at least 2 m. All sites are located within a 70 km radius of the City of Kuopio, Kuopio District, central Finland, 62 degrees 53 minutes, 32 seconds north latitude and 27 degrees 40 minutes, 56 seconds east longitude.

Crayfish populations can be characterized as sparse in the three ponds and the River Kutujoki, 0–20 crayfish per trap per night with average number closer to 5 in July and August, and abundant in Lakes Liesjärvi and Suur-Laus-

järvi, where average catch exceeded 40 crayfish per trap except for molting periods. Crayfish from sites with higher conductivities, alkalinities and hardness levels were clearly harder, with more intensively mineralized exoskeletons, than those with lower conductivities.

Weight was measured by placing live crayfish on absorbant paper for several minutes before weighing them to the nearest 0.1 g. Length measurements included: total length—distance from tip of rostrum to tip of telson with the crayfish placed on its back, nearest 1.0 mm and carapace length—tip of rostrum to postero-medial point at the distal end of the carapace, nearest 0.1 mm. Data were consolidated with an Apple Mackintosh Plus personal computer using Cricket Graphic and Cricket Statistical programs. These programs permitted comparisons of data sets using student's "t" test.

#### RESULTS AND DISCUSSION

##### Population Structure

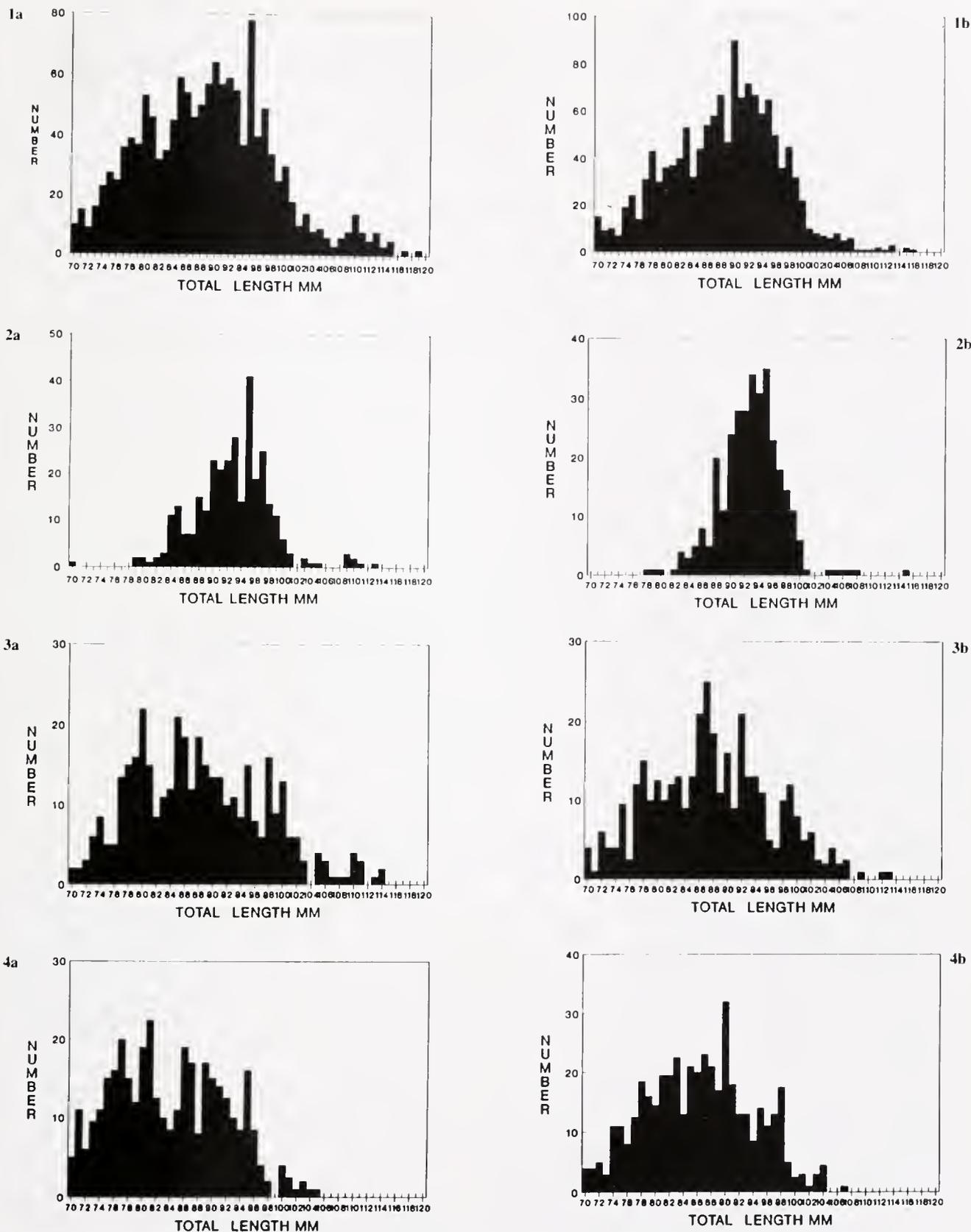
Composite total length frequency diagrams for trap caught male and female crayfish, 1987–1989, are presented in figures 1a and 1b. Size range is 7–12 cm. Very few crayfish larger than 12 cm or smaller than 7 cm were recorded from trap catches. Individual data for Lake Suur-Lausjärvi, Lake Liesjärvi, and North Valkealampi Pond are presented in figures 2a and 2b, 3a and 3b, and 4a and 4b, respectively. Very few crayfish reach the legal size of 10 cm and more males than females exceed 10 cm. Crayfish are most abundant around 9 cm and their numbers decline precipitously thereafter. Modal lengths generally suggest molt increments in the 5–7 mm range. Our own re-

TABLE 1.  
Study Sites and Characteristics for Population Studies of Noble Crayfish Populations in Central Finland.

Site	Lake Suur-Lausvasjärvi	Lake Liesjärvi	North Valkealampi Pond	South Valkealampi Pond	Theater* Pond	River Kutujoki
Size, ha	603	326	7	5	8	About 30 km long, site about 2 km long in mid-river
Maximum Depth, m	31	7	5	12	9	1 m in study area maximum 3 m, study area 15 m wide
Conductivity ohm/sq.cm.**	29–31	32–33	90–198	119–164	203–257	48–52
pH	6.2–7.1	6.9	6.7–7.1	6.8–7.1	6.9–8.5	6.9
Total Hardness, mg/l	14.2	12.8	37.4	62.3	99.7	3.0
Total Alkalinity mm./l	0.12	0.11	0.56	0.8–1.0	1.6–1.9	7.0
Location in*** Relation to Kuopio	20 km SW	40 km NW	10 km SW	10 km SW	In City	70 km SW-connects Lake Koskelavesi and Pieksanjärvi

\* Also identified as Valkeinen Pond; \*\*Corrected to 25 degrees C.

\*\*\* 62 degrees 53 minutes, 32 seconds north latitude and 27 degrees 40 minutes, 56 seconds east longitude.



Figures 1a, 1b, 2a, 2b, 3a, 3b, 4a, and 4b. Length-Frequency Data for *Astacus astacus* Caught in Traps in Various Water Bodies in Central Finland. 1a. Composite, All Males, 1987–89; 1b. Composite, All Females, 1987–89; 2a. Lake Suur-Lauasjärvi, All Males, 1988–89; 2b. Lake Suur-Lauasjärvi, All Females, 1988–89; 3a. Lake Liesjärvi, All Males, 1987; 3b. Lake Liesjärvi, All Females, 1987; 4a. North Valkealampi Pond, All Males, 1987–88–89; and 4b. North Valkealampi Pond, All Females, 1987–88–89.

TABLE 2.  
Characterization of Trap Caught Noble Crayfish from Various Populations in Central Finland.

Site mm	Years	No.	Weight g	Total Length cm	Carapace Length mm
Overall	1987, 88, 89				
Male		1437	24.1 (10.7) *	8.7 (1.1)	45.9 (6.4) *
Female		1448	20.9 (8.6)	8.8 (1.1)	44.6 (5.8) <sup>§</sup>
Lake Suur-Lauasjärvi	1988, 89				
Male		372	19.7 (6.7)	8.4 (0.8)	44.4 (4.6)
Female		441	19.0 (5.4)	8.6 (0.8)	44.4 (4.1)
Lake Liesjärvi	1987				
Male		321	28.6 (6.4) *	9.3 (0.6)	48.9 (3.2) *
Female		316	25.1 (4.7)	9.3 (5.0)	47.2 (2.8)
North Valkealampi Pond	1987, 88, 89				
Male		411	23.2 (10.0) *	8.8 (1.0)	45.5 (5.7) *
Female		367	20.3 (6.3)	8.8 (8.7)	44.4 (4.6)
South Valkealampi Pond	1987, 88				
Male		80	21.5 (7.5)	8.6 (0.9)	44.9 (5.1)
Female		135	8.7 (5.4)	8.6 (0.8)	43.4 (4.2)
Theater Pond	1987, 88, 89				
Male		173	29.1 (13.1) *	9.1 (1.1)	47.7 (6.3) *
Female		92	23.8 (8.9)	9.0 (1.1)	45.8 (5.4)
River Kutujoki	1989, 90				
Male		58	46.3 (16.4)	10.7 (1.0)	56.2 (6.1)
Female		39	47.8 (19.0)	11.4 (1.5)	57.6 (7.7)

\* Differences significant,  $P < 0.05$ —student's "t" test; Means and standard deviations in parentheses.

cordings of molts of field caught and laboratory reared crayfish from these populations agree with this conclusion. Although not presented here, consolidation of total length frequency data for 1990 through 31 August follows the same trends from the 1987–89 period both in general and within individual populations.

Summary data on mean weights, total lengths and carapace lengths are presented in Table 2. Mean total lengths range from 8.4 cm for males in Lake Suur-Lauasjärvi to 11.4 cm for females in River Kutuoki. Mean carapace lengths range from 43.4 mm in females from South Valkealampi Pond to 57.6 mm for females from River Kutujoki. Weights range from 18.7 g for females in South Valkealampi Pond to 47.8 g for females from River Kutujoki. Although the lengths of males and females are generally similar, sexual dimorphism is clear with males weighing

significantly more with the exceptions of Lake Suur-Lauasjärvi where the difference approached significance ( $P = 0.106$ ) and River Kutuoki where there was no significant difference in weight even though females were significantly larger than males, 11.4 cm versus 10.7 cm. This differential in weight is to be expected based on the well known weight difference between mature male and female *A. astacus* (Lindqvist and Lahti 1983). Largest crayfish (weight and length) were collected in River Kutuoki whose crayfish population is atypical of the situation in central Finland with a very few, very large crayfish.

The Lake Liesjärvi data fit the general pattern for composite data and specific cases of Lake Suur-Lauasjärvi and North Valkealampi Pond but the existence of various modes ("year classes") is not so apparent. We have, in fact, data for only one season, 1987, from Lake Liesjärvi.

This lake had an expanding population of crayfish that appeared about 10 years after an *A. astaci* plague episode destroyed a formerly thriving commercial fishery. Ironically, a reoccurrence of *A. astaci* plague in August 1987 again destroyed the population preventing us from obtaining further data from this lake. No crayfish have captured in test trappings in 1988, 1989, or 1990 with the exception of a few crayfish stocked to re-establish the population.

We are uncertain about the status of the Lake Liesjärvi crayfish in 1987. It may represent a relatively young population that could be expected to continue to survive and grow in significant numbers to sizes over 10 cm. Conversely, it could be a relatively old population about to experience significant mortality. The total length frequency graph for males fits the general high mortality pattern more so than does the one for females. This is due, in part, to sale of males larger than 10 cm prior to our access to samples, but it must be emphasized that absolute numbers of legal size crayfish was not high. The generally larger sizes of Lake Liesjärvi crayfish compared to other populations (Table 2) can be accounted for by the fact that the crayfish were caught with regular commercial traps. These have a larger mesh than our test traps or those used by fisherman in Lake Suur-Lauasjärvi.

Based on our data, we feel that natural mortality over the 9–10 cm size interval is high. Noble crayfish mature in the 7–8 cm size range at ages of 4–6 summers (see references in introduction). Males may then molt twice in a summer if it is particularly warm in central Finland while females rarely molt more than once. Because the size increase per molt is 5–7 mm in the populations with which we are working, an 8 cm 4th or 5th summer female crayfish would have completed the summer molt in its 6th or 7th summer upon reaching or exceeding 9 cm. This female would spawn at 8 cm, rest the following summer at a size of 8.5 cm and spawn again by the beginning of the 6th or 7th summer when she reaches or exceeds 9 cm after releasing young in early-mid summer. Maturation occurs over sizes of 7–8 cm (smallest egg bearing female collected being 6.8 cm). When coupled with favorable/unfavorable environmental conditions, this would generate the multiple modal sizes seen in our data.

Male noble crayfish also mature at sizes as small as 7 cm and all mature by 8 cm. They are reproductively active each year. The energy requirements for generating spermatophores are certainly much less intense than those required by females to generate viable ova. The 5–7 mm molt size increases for males maturing over a 7–8 cm size range and in multiple summer molts account for both observed modal groupings in the 8–9 cm size range and the presence of more males than females greater than 10 cm.

We believe that the disappearance of male and female crayfish over the 9–10 cm size range is the result of age related senility. Both males and females have engaged in reproductive activities at least twice, perhaps more times,

over the 7–9 cm size range. We have observed mid-winter mortality of summer caught male and female, 9–11 cm crayfish from North Valkealampi Pond and Lake Suur-Lauasjärvi held in our laboratory under simulated field conditions on several occasions. This could not be explained by lack of food or poor water quality. We dismissed this originally as stress related mortality but, in retrospect, it may have been associated with a senility response.

The disappearance of crayfish after they have reproduced once, rather than twice, is the rule rather than the exception in both warm and cool/water cambarid and astacid crayfishes in North America (see Payne 1978 and Hogger 1988 for reviews of crayfish life histories). Likewise, review of data presented by Svärdson (1949) and Abrahamsson (1966) for Swedish *A. astacus* populations can lead to the same conclusion. Longer life at high latitudes, at least in *A. astacus*, is apparently the result of long, very cold quiescent periods. Noble crayfish experience temperatures in the 1–4 C range from October into May of each year and relatively low temperatures even in many summers.

The disappearance of crayfish in the 9–10 cm range cannot be attributed to intense fishing pressure as has been suggested for Norwegian noble crayfish populations (Qvenild and Skurdal 1988) or predators. Observations by SCUBA divers in Lake Suur-Lauasjärvi and North Valkealampi Pond show no areas with large crayfish that do not enter traps. There is no illegal market for crayfish smaller than 10 cm in Finland. Legal markets for restocking small crayfish into other waters are not significant relative to the crayfish populations affected. An underground cottage fishery for smaller crayfish may exist but is not known to the authorities even on an unofficial basis. Furthermore, predators, if they were a significant factor, would more likely prey on smaller than larger crayfish (Stein and Magnuson 1976).

We did not distinguish between fishing and natural mortality in our study. Fishing mortality, however, is very limited at all study sites. Few crayfish reach the 10 cm legal size limit and, while smaller crayfish may, by permit, be sold for restocking purposes, their value is 20% that of legal crayfish reducing the incentive to catch them. We have seen no dramatic increase or decrease in legal crayfish over the 3–8 seasons that we have studied the populations in question.

Crayfish smaller than 9 cm are not well represented in our samples. That is, the greatest number of crayfish caught are around 9 cm. This suggests that our gear is selective with larger crayfish inhibiting smaller crayfish from entering traps. This seems to be the rule rather than the exception in crayfish populations (Abrahamsson 1966, Westin and Gydemo 1988).

Morgan and Momot (1988), building on earlier studies and reviews of crayfish life histories and energetics by

Momot et al. (1978) and Momot (1984), are of the opinion that size limits for north temperate crayfishes are needless if females are permitted to spawn at least once and are not harvested before they have released their young in the year of harvest. Furthermore, they have presented data to show that unexploited crayfish populations become dominated by large numbers of maturing males that prey on young reducing their survival and recruitment to the reproductive pool. As a result, production of useable crayfish, albeit at a size smaller than maximum for the specific habitat, is further reduced. They also speculate that a male directed fishery with a size limit, such as that favored in Scandinavia, will lower recruitment and yield because of the time delay negative feedback on hatching survival. These factors determine the maximal limit of the next year's recruitment potential to the exploitable stock. Maturing males inhibit the survival of both young-of-the-year males and females. Removal of males would favor females but only because it is a male-only fishery. Thus, some increase in the female population as a proportion of the total population would occur.

#### MANAGEMENT CONSIDERATIONS

Based on the data that we have presented and our review of pertinent literature, we feel that it would be prudent to reconsider and change the existing crayfish size limit in Finland from 10 to 9 cm for well-established, non-expanding populations. An argument that this would lead to stock collapse appears groundless because all females would have spawned young before becoming vulnerable to the fishery. Survival of those young should increase rather than decrease as a consequence of reduction of predation pressure by large males. While we do not advocate the elimination of all size limits on noble crayfish populations, it seems unlikely that consumers would accept crayfish smaller than 9 cm. Thus, first and/or second spawners would probably be protected by convention rather than by statute if size limits were eliminated. Finally, in the case of rapidly expanding populations recovering from plague episodes such as the Lake Liesjärvi population (?), females would certainly spawn once before reaching 9 cm. Fishermen would then have two options, harvest crayfish or allow them to grow to a larger size. Because many of the

relatively "young" crayfish might reach 10 cm, the fishermen could voluntarily wait until that cohort was larger and, presumably more valuable, before harvesting it. However, the ever present risk of crayfish plague episodes might be considered to be too great to wait for later profits. Market forces resulting from increases in supply from the smaller size limit would weigh heavily when such decisions were made. Fishermen could opt to harvest and sell more, lower value crayfish or risk loss of crayfish to a plague episode and harvest fewer, high value large crayfish in later summers.

The North American signal crayfish, *Pacifastacus leniusculus*, has been established in some areas of southern Finland because it is a cold water species that is normally resistant to *A. astaci* (Westman et al. 1990, Westman 1975). This species grows rapidly in favorable habitats and females may exceed 9 cm before spawning. Thus, it might be argued that broodstock would be overfished in new populations if the size limit for crayfish were reduced to 9 cm. However, the two species differ significantly in appearance and may be managed independently, if necessary. Furthermore, stabilized populations of *P. leniusculus* in Sweden are beginning to exhibit slower growth and maturation at smaller sizes (A. Fjälling, personal communication, Institute for Freshwater Research, Drottningholm, Sweden).

Some populations of Finnish *A. astacus* produce sustained catches of 10 cm crayfish of 8–21% of all crayfish larger than 7 cm (Westman and Pursiainen 1982). However, fewer females than males reach the legal size in such populations emphasizing that growth is slower and mortality higher for females, regardless of population condition.

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## LENGTH-LENGTH AND LENGTH-WEIGHT CHARACTERIZATIONS OF NOBLE CRAYFISH, *ASTACUS ASTACUS* L. (DECAPODA, ASTACIDAE), FROM CENTRAL FINLAND

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**ABSTRACT** Consolidated regression equations for length-length and length-weight relationships for noble crayfish from several populations are presented. Sexual dimorphism is apparent in mature crayfish. Males are heavier because chelae are much larger. Females have much broader abdomens to facilitate egg incubation.

**KEY WORDS:** freshwater crayfish, length-length and length-weight relationships, *Astacus astacus*, Finland

### INTRODUCTION

The noble crayfish, *Astacus astacus* L., is the dominant macrobenthic invertebrate in many Finnish freshwater ecosystems and is the subject of significant sports and commercial fisheries (Huner et al. 1991). We have studied several noble crayfish populations in central Finland for 2–8 years. We report here consolidated regression equations characterizing the length-length and length-weight relationships for these populations. These should prove useful to field workers without access to sophisticated measuring equipment.

### MATERIALS AND METHODS

Crayfish were collected in the vicinity of Kuopio, Finland. Descriptions of the study sites and population dynamics is presented elsewhere (Huner et al. 1991).

The overall size range for the crayfish measured was 4–13 cm total length (TL). The majority of the crayfish were mature, 7–11 cm TL.

Weight was measured by placing live crayfish on absorbant paper for several minutes and then weighing them to the nearest 0.1 g. Length measurements included: total length—distance from tip of rostrum to tip of telson with the crayfish placed on its back, nearest 1.0 mm; carapace length—tip of rostrum to postero-medial point at the distal end of the carapace, nearest 0.1 mm; postorbital carapace length—posterior margin of the orbit at the postero-medial point at the distal end of the carapace, nearest 0.1 mm; carapace width—widest lateral distance across the carapace taken dorsally, nearest 0.1 mm; and abdominal width—lateral distance across the second abdominal segment, taken dorsally, nearest 0.1 mm.

Data sets for total length-weight and carapace length-weight regressions are strongest in terms of total numbers, over 1,000; however, all regression equations are based on

data sets of a minimum of 150 observations over the entire size range considered and most individual populations. Male and female data were taken separately and are presented in that manner. Over 98% of the crayfish weighed and measured were intact. We included crayfish missing chelae or with small, regenerating chelae in our data sets because we wanted to provide a picture of the overall noble crayfish population in central Finland as it exists, not as it might exist if all crayfish were intact with symmetrical chelae, a biologically unrealistic situation. Total and carapace lengths were not taken from crayfish with rostrums that were broken or were obviously regenerating.

Data were consolidated with an Apple Mackintosh Plus personal computer using Cricket Graphic and Cricket Statistic programs.

### RESULTS AND DISCUSSION

Length-length and length-weight regressions are presented in Table 1. All R-values exceed 0.95 with the exceptions of the various abdominal width regressions for female crayfish. Length-length relationships have slightly higher R-values than length-weight relationships as would be expected because of the inclusion of crayfish with missing and/or small chelae as well as crayfish having different condition coefficients from different populations (Lahti and Lindqvist 1983, Lahti 1988). Both carapace width and abdominal width can be used to predict weight with a high degree of confidence. We collected postorbital carapace-carapace length data to permit conversion of postorbital carapace length, albeit somewhat indirectly, to weight. This was an unfortunate after thought as we had simply not collected postorbital carapace length-weight data routinely. Note that length-length relationships are best described by simple, linear regressions while length-

TABLE 1.

Selected composite length-length and length-weight relationship for noble crayfish from central Finland, size range 4–13 cm total length.

1. Carapace Length mm (x) – Total Length mm (y)		
Male (N = 1442) $y = 6.4803 + 1.7614x$ ;		R = 0.98
Female (N = 1448) $y = 2.606 + 1.9021x$ ;		R = 0.98
2. Postorbital Carapace Length mm (x) – Carapace Length mm (y)		
Male (N = 169) $y = 2.9223 + 1.2541x$ ;		R = 1.00
Female (N = 162) $y = 3.1915 + 1.248x$ ;		R = 1.00
3. Carapace Width mm (x) – Total Length mm (y)		
Male (N = 427) $y = 17.9337 + 3.0371x$ ;		R = 0.98
Female (N = 365) $y = 13.0805 + 3.3338x$ ;		R = 0.98
4. Carapace Width mm (x) – Carapace Length mm (y)		
Male (N = 420) $y = 7.4987 + 1.7089x$ ;		R = 0.97
Female (N = 365) $y = 6.1721 + 1.7529x$ ;		R = 0.97
5. Abdominal Width mm (x) – total Length mm (y)		
Male (N = 332) $y = 11.3652 + 3.9088x$ ;		R = 0.98
Female (N = 273) $y = 22.0617 + 2.9043x$ ;		R = 0.95
(over 70 mm TL)		
Female (N = 54) $y = 13.4222 + 3.5777x$ ;		R = 0.97
(under 70 mm TL)		
6. Abdominal Width mm (x) – Carapace Length mm (y)		
Male (N = 332) $y = 3.7132 + 2.2236x$ ;		R = 0.98
Female (N = 273) $y = 10.4884 + 1.5504x$ ;		R = 0.95
(Over 70 mm TL)		
7. Total Length mm (x) – Weight g (y)		
Male (N = 1442) $y = 0.4976 * 10' (0.0187x)$ ;		R = 0.98
Female (N = 1448) $y = 0.6159 * 10' (0.0171x)$ ;		R = 0.97
8. Carapace Length mm (x) – Weight g (y)		
Male (N = 1442) $y = 0.6297 * 10' (0.0328x)$ ;		R = 0.97
Female (N = 1448) $y = 0.6601 * 10' (0.0328x)$ ;		R = 0.96
9. Carapace Width mm (x) – Weight g (y)		
Male (N = 420) $y = 0.881 * 10' (0.0609x)$ ;		R = 0.97
Female (N = 370) $y = 0.7224 * 10' (0.0641x)$ ;		R = 0.98
10. Abdominal Width mm (x) – Weight g (y)		
Male (N = 377) $y = 0.6227 * 10' (0.0795x)$ ;		R = 0.98
Female (N = 335) $y = 1.0493 * 10' (0.0564x)$ ;		R = 0.97

weight relationships are better described by exponential regressions.

Our length-length and length-weight regression equations are generally in agreement with our earlier individual reports for one of our study populations in North Valkea-

lampi Pond (Huner et al. 1988) and other literature available for *A. astacus* from central Finland (Lindqvist and Lahti 1983). Note that our data provide information over a broader size range, 4–13 cm, than previously published.

A clear distinction could be demonstrated between abdominal width of females less than 7 cm total length and those above it as a consequence of the widening of the abdomen at maturation to permit incubation of fertilized eggs. The ratio of carapace width to abdomen width was 1.112 (SD = 0.042, N = 54) for females smaller than 7 cm and 0.997 (SD = 0.048, N = 281) for larger females ( $t = 16.485$ ,  $P < 0.001$ ). Likewise, the width of female abdomens was much greater than those of males, ratio of carapace length to abdominal width being 1.016 (SD = 0.063, N = 335) and 1.173 (SD = 0.048, N = 337) ( $t = 36.273$ ,  $P = 0.001$ ). The ratio of carapace length to carapace width was 2.078, (SD = 0.097, N = 332) in males and 2.065 (SD = 0.090, N = 327) in females ( $t = 1.714$ ;  $P = 0.087$ ). This is indicative of a narrower carapace in males. The ratio of total length to carapace length was 1.905 (SD = 0.051, N = 1437) for males and 1.964 (SD = 0.052, N = 1448) ( $t = -30.059$ ,  $P = 0.001$ ) showing that male carapace length was greater than that of females.

While we could provide individual population regressions, we feel that composite regressions would be of greater value to those working with *A. astacus* populations where it is impractical to weigh crayfish. Furthermore, total length, although not an appropriate measure for taxonomic purposes can certainly be used to project crayfish weights with reasonable accuracy and can be related to other, more stable body measurements.

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**PROCEEDINGS OF THE SPECIAL SYMPOSIUM: REPRODUCTIVE BIOLOGY  
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## NEGATIVE LARVAL RESPONSE TO SELECTION FOR INCREASED GROWTH RATE IN NORTHERN QUAHOGS *MERCENARIA MERCENARIA* (LINNAEUS, 1758)

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**ABSTRACT** F<sub>1</sub> offspring of wildstock *Mercenaria mercenaria* from Georgia were subjected to truncation selection (16% intensity level). Larval (F<sub>2</sub>) progeny of *M. mercenaria* selected for rapid growth rate were significantly smaller (shell length) than larval progeny of control parents at both 10 and 18 days of age in two experimental trials. Survival rates were similar for both progeny lines from 2 to 18 days of age. Earlier studies by our group have demonstrated significantly higher embryonic mortality rates (2 days) in the progeny of parents selected for rapid growth. Control line progeny in both experiments set earlier (10–14 days) than those of the select line parents (14–18 days). This negative larval response for increased growth rate (in 3 year old adults) brings into question the merits of hatchery culling practices for smaller larvae. A long term approach to the study of the reproductive potential of bivalve brood-stock lines selected for increased rate of growth is called for on the basis of these results.

**KEY WORDS:** *Mercenaria mercenaria*, selection, larvae, aquaculture

### INTRODUCTION

The relatively limited utilization of genetic selection techniques in molluscan mariculture have been reviewed recently (Humphrey and Crenshaw 1989). Early efforts studied the heritability for increased growth in larval and spat stages, e.g., Longwell (1976), Newkirk et al. (1977), Losee (1979), and more recently Stromgren and Nielsen (1989). Newkirk and Haley (1982) reported a lack of correlation between length of larval period (and larval size) and size of juvenile and older European oysters, *Ostrea edulis* and concluded that selection for growth to marketable size for bivalves should be conducted on stocks of at least two years of age. Wada (1986) has reported on the realized heritabilities for selection for shell traits in the Japanese pearl oyster. Toro and Newkirk (1990) have recently reported heritability estimates for their efforts utilizing divergent selection (full sib families) with the European oyster. The study reported herein is the first to examine the viability and growth dynamics of larval stages propagated from F<sub>1</sub> select (and control) bivalve stocks. Truncation selection for shell length was carried out on two separate lines of *Mercenaria mercenaria*, with a selection intensity of approximately 16% in both cases. Preliminary mass culture experience during 1988 with Group A select lines (AS) and wild-stock control brood stocks suggested lower viability and growth rates among the selected line's progeny. On the basis of these experiences, the current study was designed to evaluate specifically the larval survival and growth rate dynamics of select and control lines of *M. mercenaria*. A separate study by this group evaluated similar dynamics for the embryonic phase (48 hours), and will be reported separately. This genetic selection program is part of a long term

developmental program for *M. mercenaria* mariculture in Georgia.

### MATERIALS AND METHODS

Two cohorts of *Mercenaria mercenaria* were established from separate mass spawnings of wild stock clams from House Creek, Little Tybee Island, Wassaw Sound, Georgia, USA, on April 4 (Group A) and May 8 (Group B) 1986. Post settlement (juvenile) stages were reared in downwelling nursery systems following standard nursery culture procedures for bivalve molluscs (Castagna and Kraueter 1981, Heffernan et al. 1988). However, following the principles of quantitative genetic selection (see Falconer 1981), these cohorts were never subjected to size screening, as is the common practice in commercial hatcheries (Castagna and Kraueter 1981). During December 1986, both cohorts were transferred to field growout cages in a sheltered intertidal creek (House Creek) in Wassaw Sound, Georgia (Crenshaw et al. 1988). Selection was carried out for Group A on March 16, 1988, while Group B selection was delayed until May 3, 1989, due to their relatively small size. Select and Control lines for rate of growth were established using shell length (SL) measurements for each group with the top 16% approximately in each group serving as the cut off point for selection purposes. The mean sizes of the selected parental arrays were 1.506 and 1.526 standard deviation ( $\sigma$ ) above their population means. These figures represent the standardized intensity of selection (i) for the two groups.

The Group B F<sub>1</sub> Control and Select line broodstock (N = 128 each line) were mass spawned on June 13 and 14, 1989, respectively, while Group A Control and Select line broodstock (N = 162) were similarly induced to spawn on

July 6 and 7, 1989, respectively, using thermal stimulation (Castagna and Krauter 1981). The number, sex and SL of parents spawning during each mass spawning event were recorded. Fertilized eggs were collected by filtering (20  $\mu\text{m}$ ) the water from the spawning table and egg suspensions were established in 10 L of filtered seawater. Drain-down procedures were handled similarly for all four spawnings. Five samples (1 ml) were obtained from each spawning event's egg suspension following standard mixing techniques (Castagna and Krauter 1981). These samples were immediately counted with a compound microscope (100 $\times$ ) and a mean egg density was calculated for each suspension. These eggs were then added to individual culture tanks at similar densities (20/ml) and maintained at 26°C for approximately 48 hours. After 48 hours a similar enumeration procedure for individual group larval density levels were conducted, as described above (fixed samples). A similar experimental design was subsequently employed to evaluate larval survival and growth rates for the four treatments [i.e., Group A Select (AS), Group A Control (AC), Group B Select (BS), Group B Control (BC) F<sub>2</sub> cohorts].

One L flask replicates (N = 30 per treatment) were established and appropriate volumes were added from each larval suspension to create an initial stocking density of 1/ml (1000 larvae/1 L flask). The seawater in each experimental flask was filtered at 1  $\mu\text{m}$  and maintained under relatively constant temperatures (25°  $\pm$  1°C) and salinity (28–30 ppt) in a temperature control room. In order to validate initial stocking densities, five replicates were sacrificed immediately, washed onto 20  $\mu\text{m}$  mesh sieves, then concentrated into a small volume of filtered seawater (20–30ml) and fixed (v:v) with 10% neutral buffered formalin. Fixed samples were subsequently stained with Rose Bengal. On days 6, 10, 14, 16, and 18, five additional replicates per treatment were similarly sacrificed and processed for analysis. All replicates were examined by stereomicroscope (40 $\times$ ) for evidence of metamorphosis/setting prior to fixation. Initial plans to evaluate survival on the basis of subsampling (N = 3) fixed replicate suspensions were discarded due to their inaccuracy level, especially with metamorphic stages. Survival data were gathered on the basis of total counts for each sampling day. Shell length measurements were obtained (using an ocular micrometer, 100 $\times$ ) for 30 fixed larvae in each sacrificed flask on days 2, 10 and 18 (i.e., 150 individuals each in AS; AC; BS and BC per sampling date). Seawater (1  $\mu\text{m}$  filtered) was changed in each larval flask every second day. A daily food ration of 40,000 cells/ml of *Isochrysis galbana* (Strain T.ISO) was added to each larval flask [algal cell concentrations were determined fluorometrically having previously established the relationship between fluorescence level (Y) and cell concentration (X);  $Y = -2.7298 + 3.50361X$ ;  $R^2 = 0.9576$ ]. Data analysis included the use of t-tests for initial stocking density comparisons and 2—sample

ANOVA's for pooled SL data comparisons on days 2, 10 and 18 using SPSS software.

## RESULTS

Detected spawners per F<sub>1</sub> treatment group ranged in number from 20 to 57, with males outnumbering females in three of the four events (Table 1). Mean sizes for the various parental lines (i.e., spawners) ranged from 43.7 mm SL (BC) to 52.7 mm SL (AS) (Table 1). Initial (mean) stocking density levels in the larval trials were as follows: AC = 801.0  $\pm$  69.4 (2SE); AS = 616.8  $\pm$  198.9; BC = 579.4  $\pm$  142.6; BS = 1265.8  $\pm$  191.6 (Figs. 1, 2). Differences between AC and AS initial stocking densities were not significant (t-test,  $p = 0.131$ ), whereas those between BC and BS were (t-test,  $p = 0.0021$ ). Apart from the day 2 to 6 period in BC and BS, survival rates were similar among the Select and Control treatments in both lines, with no significant differences detected among mean number of survivors from days 6 to 18 (Figs. 1, 2).

Metamorphosis took place earlier in the Controls, AC and BC larvae set between days 10–14, whereas AS and BS larvae did not do so until days 14–18. Larval SL means at the start of the experiments are portrayed in Table 2, showing AS ( $\bar{x} = 99.6 \mu\text{m}$ ) were significantly larger ( $p < 0.001$ ) than AC ( $\bar{x} = 95.1 \mu\text{m}$ ) and BC ( $\bar{x} = 98.1 \mu\text{m}$ ) were significantly larger than BS ( $\bar{x} = 93.9 \mu\text{m}$ ) ( $p < 0.006$ ). Despite the contradictory size relationships at the commencement, a clear trend in larval growth rates was established during the course of the experiments, with the Controls outgrowing the Selects in both trials (Table 2). AC ( $\bar{x} = 205.8 \mu\text{m}$ ) and BC ( $\bar{x} = 206.7 \mu\text{m}$ ) were significantly larger than AS ( $\bar{x} = 192.1 \mu\text{m}$ ) and BS ( $\bar{x} = 183.5 \mu\text{m}$ ), respectively, at day 10 ( $p < 0.0001$  in both cases) (Table 2). By day 18 the size differences had increased with AC ( $\bar{x} = 397.1 \mu\text{m}$ ) and BC ( $\bar{x} = 375 \mu\text{m}$ ) again highly significantly larger than AS ( $\bar{x} = 314.7 \mu\text{m}$ ) and BS ( $\bar{x} = 265.8 \mu\text{m}$ ), respectively, ( $p < 0.0001$  in both cases) (Table 2). The Controls (F<sub>2</sub>) progeny were 26.1% and 41.1% larger than those Selects (F<sub>2</sub>) at 18 days in Groups A and B, respectively.

TABLE 1.

*Mercenaria mercenaria* F<sub>1</sub> brood stock spawners.

		AS	AC	BS	BC <sup>1</sup>
Spawners	♂	22	14	13	23
	♀	15	6	43	11
Mean SL (mm)		52.7	45.4	48.4	43.7
SE		0.8	1.1	0.2	0.8

<sup>1</sup> AS = Group A Select; AC = Group A Control; BS = Group B Select; and BC = Group B Control.

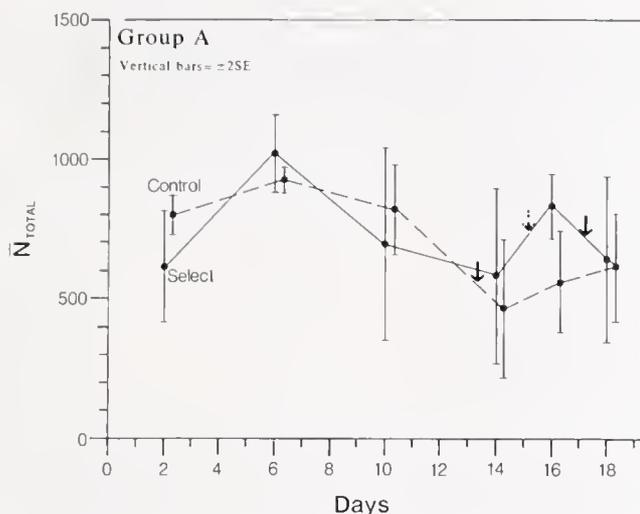


Figure 1. Survival data for Group A larval progeny (F<sub>2</sub>) from day 2-18. Solid arrows indicate metamorphosis-setting; broken arrows indicate partial metamorphosis-setting.

#### DISCUSSION

In their early studies of the potential for genetic selection studies with commercial bivalves, Newkirk et al. (1977) and Losee (1979) reported positive correlations between larval and subsequent spat growth rates. However, these studies were limited to relatively short term analyses of the spat stages (about eight months post settlement in both cases) and as Newkirk and Haley (1982) subsequently reported, the correlation between length of larval period and size of later stages is lost with time. In contrast to these earlier reports, the results presented herein display a negative correlation between adult (commercial size) growth rates and the growth rates of their larval progeny (to 18 days). This interpretation stems from our rejection of the

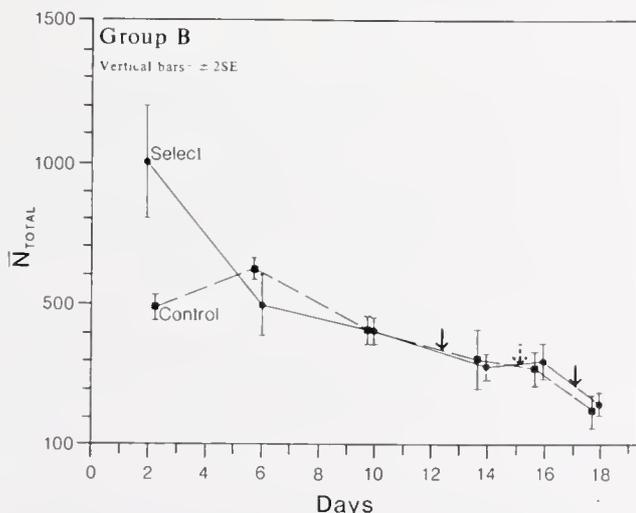


Figure 2. Survival data for Group B larval progeny (F<sub>2</sub>) from day 2-18. Solid arrows indicate metamorphosis-setting; broken arrows indicate partial metamorphosis-setting.

null hypothesis that there is no difference between the growth rates of select and control line F<sub>2</sub> larval progeny, with the latter exhibiting greater growth rates than the former. Clams selected for faster growth rates were shown to produce slower growing larvae than their control line partners when both were spawned at approximately three years of age. It is the hypothesis of the authors that the observed difference in progeny growth rates is primarily due to genetic differences between the treatments, as both Select and Control lines were treated equally throughout their respective larval, nursery, field grow out and conditioning periods. Lannan et al. 1980 reported on the importance of the conditioning regimen and subsequent larval returns for commercial hatcheries.

It would appear from the results of this study that there were no significant differences in the survival rates among the larval progeny of Select and Control line stages. The only survival differences detected during this study (i.e., among BS and BC from days 2 to 6) may have been due to the significantly higher initial stocking density in BS; however, this is thought to be unlikely as both densities were below those recommended for commercial culture (Castagna and Kraueter 1981). Another possible explanation for this difference may be that it is an extension of the embryonic stage (48 hours post fertilization) survival differences. Select line progeny had significantly lower survival rates than Controls in both Groups A and B (Heffernan et al. in prep.).

As this study has demonstrated, a negative correlation between larval and subsequent growth rates to market size exists for *Mercenaria mercenaria*. Assuming that this trend proves to be consistent among other cultured bivalve species, it is reasonable to question the merits of the larval culling practices carried out worldwide in bivalve hatcheries. It is likely, on the basis of the results presented herein and those of Newkirk and Haley (1982), that a substantial proportion of the larvae routinely discarded during hatchery cullings would turn out to be individuals which would have grown to a market size more rapidly than many of those retained by the culling practice. Of course, this theoretical discourse demands substantiation by commercial scale evaluations with several species.

The ecological implications of the results of this study are consistent with the principle of genetic homeostasis as proposed by Lerner (1954), and later discussed by Falconer (1981), "if we change any metric character by artificial selection we must expect a reduction of fitness as a correlated response." The higher mortality rates of Select line progeny during the embryonic phase (Heffernan et al. in prep.) are consistent with this principle. One may postulate that in the natural environment the Select line progeny would also suffer fitness trait reductions in the form of slower larval growth rates, longer time to metamorphosis, and smaller size at metamorphosis, all of which would probably contribute to higher susceptibility to predation.

TABLE 2.

Larval shell length (SL) data from two experimental trials comparing the offspring ( $F_2$ ) of Select and Control Groups. Group A Control and Select lines ( $F_1$ ) spawned July 6 and 7, 1989, respectively. Group B Control and Select lines ( $F_1$ ) spawned June 13 and 14, 1989, respectively.

	SL ( $\mu\text{m}$ )		Analysis of Variance		
	Mean	Range	df	F	p
Day 2					
Group A					
Select	99.6	(97.7–103.3)			
Control	95.1	(88.0–102.3)	1	13.037	<0.001
Group B					
Select	93.9	(90.7–97.3)			
Control	98.1	(93.3–101.7)	1	7.746	<0.006
Day 10					
Group A					
Select	192.1	(177.3–203.7)			
Control	205.8	(195.3–217.7)	1	20.917	<0.0001
Group B					
Select	183.5	(171.0–192.3)			
Control	206.7	(187.0–220.0)	1	65.088	<0.0001
Day 18					
Group A					
Select	314.7	(248.3–370.0)			
Control	397.1	(369.3–425.3)	1	311.241	<0.0001
Group B					
Select	265.8	(238.7–289.7)			
Control	375.0	(329.3–415.7)	1	194.935	<0.0001

However, under culture conditions where predation pressure is eliminated, we have not detected any post set mortality differences among Select and Control lines (personal observation) which could be linked to selection pressure. This is an area which suggests itself for future experimental evaluation. Other areas which demand further evaluation arising from this research are: age-, generation of selection-, and intensity of selection-specific effects of selection pressure on brood stock performance.

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## LIPIDS AND PROTEINS IN EGGS OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA* (GMELIN, 1791)) AND NORTHERN QUAHOGS (*MERCENARIA MERCENARIA* (LINNAEUS, 1758))

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**ABSTRACT** Eggs were isolated from the ovaries of *Crassostrea virginica* and *Mercenaria mercenaria*. The major materials in the eggs were protein (40–50% of the dry weight), lipid (14–21%) and carbohydrate (7–9%). *C. virginica* eggs had more lipid (21%) than *M. mercenaria* (14%) due to a higher concentration of lipid droplets. These lipid droplets were primarily composed of triglycerides. The major water-soluble protein in the eggs of both bivalve species was a very high density lipoprotein containing a 56,000 dalton peptide and phospholipid. These lipovitellins may play an important role in the developing embryo.

**KEY WORDS:** lipids, *Crassostrea virginica*, *Mercenaria mercenaria*, lipoproteins, bivalves, proteins, eggs

### INTRODUCTION

The protein, lipid and carbohydrates in bivalve eggs are important in providing energy and nutrients for the developing embryo. Egg storage lipids also play a major role in the growth and survival of bivalve larvae (Holland 1978, Chu and Webb 1984, Gallager and Mann 1986, Epp et al. 1988). Lipids within invertebrate eggs are distributed among membranes, lipid droplets and water-soluble lipoproteins. The membrane lipids have a structural function and are predominantly phospholipids and sterols. Lipid droplets have a storage function and are composed primarily of triglycerides. The water-soluble lipoproteins, which are supramolecular complexes of lipids and polypeptides, have a role in the transport of lipids between their sites of synthesis, storage and utilization. The major water soluble proteins of invertebrate eggs are referred to as vitellins. Wallace et al. (1967) used the term "lipovitellin" for the high density lipoproteins found in invertebrate eggs. These are often the major water soluble protein of the eggs. The lipovitellins of crustacean eggs are composed of phospholipids, cholesterol, and high molecular weight peptides (Wallace et al. 1967, Lee and Puppione 1988, Lee 1991). While several studies have investigated the lipids of bivalve eggs (Bayne et al. 1975, Holland 1978, Gallager and Mann 1986, Gallager et al. 1986), there have been few studies to characterize the lipoproteins and other proteins of bivalve eggs. Egg lipoproteins isolated from the eggs of the scallop (*Pecten maximus*) had phospholipid as the primary lipid class (Zagalsky et al. 1967).

Our studies determined the amount of different lipid classes among lipid droplets, bivalve membranes and lipoproteins within the eggs of the eastern oyster (*Crassostrea virginica*) and hard clam (*Mercenaria mercenaria*). The

major water-soluble proteins, including lipoproteins, were characterized as to their molecular masses.

### MATERIALS AND METHODS

*Mercenaria mercenaria* and *Crassostrea virginica* were collected in coastal Georgia during their peak spawning periods which were April–May for *M. mercenaria* (Heffernan et al. 1989a) and June–July for *C. virginica* (Heffernan et al. 1989b). Eggs were washed out of sliced gonadal tissues with filtered seawater. Nylon filters were used to purify the egg preparations. A prefilter of 110  $\mu\text{m}$  was used to remove material larger than the eggs followed by a 40  $\mu\text{m}$  filter for *M. mercenaria* eggs and a 20  $\mu\text{m}$  filter for *C. virginica* eggs. Eggs were rinsed several times with filtered seawater. A portion of the eggs were taken for counting and dry weight determination. The rest of the eggs were homogenized in 100 mM TRIS buffer (pH 8.0). Portions of the homogenate were removed for protein, lipid and carbohydrate determinations. The remainder of the homogenate was centrifuged at 13,000  $\times$  g. Floating lipid droplets were removed and washed twice followed by extraction of lipids. The 13,000  $\times$  g supernatant was centrifuged at 100,000  $\times$  g for one hour to sediment microsomes. Lipoproteins were isolated from the 100,000  $\times$  g supernatant (cytosol). Cytosol lipoproteins were separated from other proteins by adjusting the cytosol density with solid KBr and ultracentrifugation at 117,000  $\times$  g at 17°C in a Beckman L5-40 equipped with a 40.3 rotor. All salt solutions used to adjust the solution density were prepared according to the methods outlined by Lindgren (1975). Solution densities were verified by refractometry using a Bausch and Lomb Abbe Refractometer. Prior to electrophoresis or analysis, isolated lipoprotein fractions were



TABLE 1.  
Composition of eggs of *Crassostrea virginica* and  
*Mercenaria mercenaria*.

Component	<i>C. virginica</i> (ng/egg)	(% dry weight)	<i>M. mercenaria</i> (ng/egg)	(% dry weight)
Dry weight	12	—	51	—
Protein	6	50	20	40
Carbohydrate	1.1	9	4	8
Lipid	2.5	21	7	14
Triglycerides	1.9		2.0	
Sterols	0.1		0.1	
Phospholipids	0.4		4.6	

dialyzed for 24 hours at 4°C against a 0.10 M NaCl buffer (pH 8.0) containing 1 mM EDTA and 2 mM sodium azide.

Lipoprotein and protein preparations were run on 7.5% polyacrylamide gels containing 0.1% sodium dodecylsulfate (SDS) and 0.8%  $\beta$ -mercaptoethanol; using the procedures of Laemmli (1970). Molecular weights of the peptides were determined using a series of proteins of known molecular weight (Weber and Osborn 1969). Stained gels were scanned at 595 nm with an E-C densitometer equipped with a peak integrator.

Protein concentrations were determined by the method of Bradford (1976). Carbohydrates were determined by the method of Dubois et al. (1956). Lipids were extracted by the procedure of Folch et al. (1957) and quantified gravimetrically. Different lipid classes were separated on silica-coated chromatopods and quantified on an Iatroscan Mark IV equipped with a flame ionization detector. Cholesterol, cholesteryl oleate, oleic acid and triolein were the reference standards.

## RESULTS

*C. virginica* and *M. mercenaria* eggs differed in dry weights being 12 and 51 ng/egg, respectively (Table 1). The difference between the dry weight and the organic constituents (protein, lipid, and carbohydrate) is assumed to be ash. The predominant egg constituent was protein (40–50% of the dry weights). *C. virginica* eggs had 21% lipid versus 14% for those from *M. mercenaria*.

TABLE 2.  
Distribution of lipid within eggs of *Crassostrea virginica* and  
*Mercenaria mercenaria*.

Fraction	Lipid Concentration	
	<i>C. virginica</i> (ng/egg)	<i>M. mercenaria</i> (ng/egg)
Lipid Droplets	1.6	1.5
Membranes	0.7	4.8
Lipoproteins	0.2	0.6

Egg lipids included triglycerides, sterols and phospholipids. These lipids were associated with oil droplets, membranes and water-soluble lipoproteins found within the eggs (Table 2). Analysis of oil droplets revealed them to be



Figure 1. Electrophoresis of egg proteins from *C. virginica* and *M. mercenaria* on 7.5% polyacrylamide gel in dodecylsulfate. Samples A and B were run at different times and on different gels from C and D. They are juxtaposed for this figure and include: Lane A, cytosolic proteins from eggs of *C. virginica*; Lane B, standards: muscle phosphorylase (97,400), albumin (66,200), carbonic anhydrase (31,000), trypsin inhibitor (21,500), lysozyme (14,400), Lane C, cytosolic proteins from eggs of *M. mercenaria*; and Lane D, standards: muscle myosin (200,000), galactosidase (116,000), phosphorylase (97,400), albumin (66,200), ovalbumin (42,700), carbonic anhydrase (31,000), trypsin inhibitor (21,500), lysozyme (14,400).



composed almost entirely of triglycerides (98%) with traces of sterols, phospholipids and protein. The relative oil droplet content was higher in *C. virginica* than in *M. mercenaria* and thus, triglycerides accounted for 76% of the lipid in *C. virginica* eggs and only 30% in *M. mercenaria* eggs (Tables 1 and 2). The predominant lipid of the membrane fraction and lipoproteins were phospholipids ( $\approx 80\%$ ) with small amounts of sterols and steryl esters. Thus, most phospholipids were associated with various membranes within the egg.

Proteins associated with the cytosol, which was the supernatant from  $100,000 \times g$  centrifugation, accounted for approximately 80% of the total egg protein. The major water-soluble peptide, i.e., cytosolic proteins, of both *C. virginica* and *M. mercenaria* was a peptide with a molecular mass of 56,000 daltons (Fig. 1). Other important egg peptides in *C. virginica* had molecular masses of 76,000, 50,000, 48,000, 18,000, and 17,000 daltons. The major egg peptides of *M. mercenaria*, besides the 56,000 dalton band, had masses of 98,000, 87,000, 68,000, 60,000, 36,000 and 19,000 daltons. The 56,000 dalton band accounted for 85% and 60% of the water-soluble proteins in *M. mercenaria* and *C. virginica*, respectively. The 56,000 dalton peptide was associated with a very high density lipoprotein (density: 1.21–1.31 g/ml). The lipoprotein was 80% protein, 17% lipid and 2% carbohydrate.

#### DISCUSSION

Bivalve eggs are characterized by numerous lipid droplets (Gallager and Mann 1986). As shown by our studies, these lipid droplets were predominantly triglycerides and are possibly similar in structure to the triglyceride-rich chylomicrons found in vertebrate blood. The amount of lipid and the type of lipids we found in *C. virginica* and *M. mercenaria* are similar to those reported for these same species by Gallager et al. (1986). The higher concentration of triglyceride in *C. virginica* compared with *M. mercenaria* suggests that *C. virginica* larvae can survive longer after hatching before feeding is required for

survival. The relative amount of protein (40–50%), lipid (14–21%), and carbohydrate (7–9%) we found in *C. virginica* and *M. mercenaria* were similar to the relative amounts of these materials in the eggs of other bivalve species (Bayne et al. 1975, Holland 1978, Whyte et al. 1987).

The predominant water-soluble protein of both oysters and clams was a very high density lipoprotein characterized by phospholipid and a 56,000 dalton peptide. Recently, we found that the major protein of the bay scallop, *Argopecten irradians concentricus*, was also a 56,000 dalton peptide (Lee, unpublished data). We are not aware of other studies which have characterized the major protein of bivalve eggs.

The primary peptide in crab and lobster ovaries had a molecular mass of approximately 100,000 daltons while a second peptide, which was often present, was approximately 80,000 daltons (Lee and Puppione 1988, Lee 1991). Crustacean egg lipoproteins are much higher in lipid content (40–50%) than bivalve egg lipoprotein. Thus, it appears that the lipovitellin of crustacean and bivalve eggs are quite different in composition and size. Further studies should be carried out to determine the importance of bivalve lipovitellin for the developing embryo. We are presently carrying out studies to further characterize the 56,000 dalton peptide found in bivalve eggs.

The sequence of events which take place in the female to form the lipids and lipoproteins found in the eggs and how these compounds are utilized by the developing embryo should help in the development of bivalve aquaculture. For example, if culture conditions are not optimal, then insufficient lipovitellin may be transferred to the eggs resulting in poor viability after hatching.

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## ELIMINATING SPAT SETTLEMENT ON OYSTERS CULTURED IN COASTAL GEORGIA: A FEASIBILITY STUDY

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**ABSTRACT** This study was designed to evaluate site of deployment and bag rotation as antifouling (oyster spat) methods to be used in conjunction with oyster bag culture. In addition to monitoring spatfall per oyster, growth and survival rates of cultured oysters were recorded. Replicate growing bags (n = 3), each containing 75 oysters ( $\bar{x}$  shell length (SL) = 41.9 ± 1 mm/replicate), were deployed subtidally (on the river bottom and off-bottom on a trestle) and intertidally (on the bottom) in December 1988. Bags were rotated either seasonally, once monthly, or twice monthly. Oyster spat settlement was significantly higher on oysters placed subtidally off-bottom ( $\bar{x}$  = 26.7 spat/oyster) than on those placed on intertidal ( $\bar{x}$  = 0.9 spat/oyster) or subtidal ( $\bar{x}$  = 0.2 spat/oyster) bottoms. Final SL (Sept 1989) was significantly greater for oysters grown subtidally off-bottom ( $\bar{x}$  SL = 64.5 mm). Subtidal bottom oysters ( $\bar{x}$  SL = 58.8 mm) exhibited a larger final shell length than those oysters placed intertidally on the bottom ( $\bar{x}$  SL = 54.2 mm). Subtidal off-bottom oysters exhibited the greatest amount of survival ( $\bar{x}$  % survival = 68.0), and subtidal bottom treatments tended to have the lowest survival rates ( $\bar{x}$  % survival = 26.0). Bag rotation had no effect on spat settlement, growth, or survival rates of the cultured oysters. Fouling by oyster spat on oysters being cultured in a field grow out system can be effectively eliminated by placing oysters on the intertidal bottom during the spawning season (May–Nov), and maximum growth rates maintained by placing the oysters subtidally off-bottom during the non-spawning season.

**KEY WORDS:** oysters, spat settlement

### INTRODUCTION

Culture of the oyster *Crassostrea virginica* (Gmelin 1791) in the coastal waters of Georgia exhibits advantages over similar culture in more northern latitudes of the eastern United States. Continuous growth of oysters occurred in Georgia during winter months (Heffernan and Walker 1988), when oysters in the more northern areas normally exhibited no growth (Shaw 1968, Matthiessen and Toner 1966). Southern areas also tended to promote greater instantaneous growth rates for oysters than northern regions. An 18 month growth period was postulated for a market sized (76.2 mm SL) oyster grown off-bottom in Georgia (Heffernan and Walker 1988) compared to 3 years in Maryland (Beaven 1953) and Martha's Vineyard, Massachusetts (Matthiessen and Toner 1966) and 2.5 years in Chatham, Massachusetts (Shaw 1968) for raft grown oysters.

The spawning season of oysters in Georgia ranges from May to October/November (Heffernan et al. 1989), with the peak occurring from July to September. Presently no studies exist which delineate oyster spat settlement patterns within the intertidal zone for the coastal waters of Georgia, where the majority of oyster beds are found (Harris 1980); however, in South Carolina, McNulty (1953) found spat

settlement to be heaviest 0.3 m above the mean low water mark for inshore areas, whereas heaviest settlement occurred below the mean low water mark in waters nearer the Atlantic Ocean.

The state of Georgia has the potential to be a leading producer of *C. virginica*, which is evident by the fact that Georgia led the nation in oyster production in 1908 with over 3.5 million kgs of oyster meats being harvested

TABLE 1.

Rotational regimen and area of deployment of oysters which were placed in 13 mm mesh growing bags (n = 75/bag) and deployed into Skidaway River, Georgia.

Area	Rotation
Intertidal bottom	* Seasonally
Intertidal bottom	Once monthly
Intertidal bottom	Twice monthly
Subtidal bottom	* Seasonally
Subtidal bottom	Once monthly
Subtidal bottom	Twice monthly
Subtidal off-bottom	* Seasonally
Subtidal off-bottom	Twice monthly

\* Seasonally = Trimonthly

TABLE 2.

Mean number of spat settled on oysters placed in 13 mm mesh oyster growing bags (75/bag when deployed initially in Dec 1988) in Skidaway River, Georgia and rotated at various times. Subsets (N = 30/bag) were examined trimonthly and no spat were detected until the June 1989 sampling date. Mean number of spat are given  $\pm$  one standard error (SE).

Area/Monthly Rotation	June 1989		September 1989	
	S	N	S	N
<i>Intertidal Bottom</i>				
Seasonal				
1	3.5 $\pm$ 0.83	30	5.1 $\pm$ 2.26	30
2	1.1 $\pm$ 0.50	30	0.4 $\pm$ 0.18	30
3	1.3 $\pm$ 0.47	30	0.4 $\pm$ 0.11	30
Once				
1	0.4 $\pm$ 0.21	30	0.3 $\pm$ 0.18	30
2	1.9 $\pm$ 0.50	30	0.8 $\pm$ 0.31	30
3	1.6 $\pm$ 0.57	30	0.4 $\pm$ 0.22	30
Twice				
1	0.4 $\pm$ 0.12	30	0.3 $\pm$ 0.16	30
2	0.1 $\pm$ 0.08	30	0.1 $\pm$ 0.11	30
3	0.4 $\pm$ 0.21	30	0.3 $\pm$ 0.10	30
<i>Subtidal Bottom</i>				
Seasonal				
1	1.4 $\pm$ 0.41	30	0.1 $\pm$ 0.09	16
2	0.3 $\pm$ 0.16	30	0.0 $\pm$ 0.06	17
3	1.8 $\pm$ 0.57	30	0 $\pm$ 0	12
Once				
1	Lost		Lost	
2	Lost		Lost	
3	Lost		Lost	
Twice				
1	1.3 $\pm$ 0.30	30	0.1 $\pm$ 0.05	30
2	2.2 $\pm$ 0.57	24	0.2 $\pm$ 0.11	16
3	0.8 $\pm$ 0.27	30	0.4 $\pm$ 0.23	27
<i>Subtidal Off Bottom</i>				
Seasonal				
1	21.8 $\pm$ 0.83	30	23.7 $\pm$ 1.97	30
2	24.7 $\pm$ 0.50	30	24.2 $\pm$ 2.30	30
3	29.6 $\pm$ 0.47	30	32.9 $\pm$ 2.32	30
Twice				
1	29.5 $\pm$ 0.12	30	24.6 $\pm$ 2.21	30
2	22.5 $\pm$ 0.08	30	25.3 $\pm$ 1.95	30
3	29.8 $\pm$ 0.21	30	29.2 $\pm$ 1.86	30

S =  $\bar{x}$  no spat  $\pm$  SE

N = No. of oysters sampled

(Harris 1980). The industry has since experienced a devastating decline, reaching a low of 1,787 kgs of oyster meats harvested in 1986 (Gordon Rogers personal communication). The decline is apparently the result of a combination of factors, the foremost being mismanagement by over harvesting and not returning suitable cultch material to commercial beds for future spat collection (Linton 1968, Harris 1980, Ofiara and Stevens 1987). Other contributing factors include pollution (Carley and Frisbie 1968), disease (Harris 1980), and economics (Ofiara and Stevens 1987).

One of the major problems confronting the industry today is overcrowding due to the plethora of oyster larvae produced during the spawning season and the lack of proper settlement sites. This results in densely packed

clumps of oysters which are capable of only lateral growth. These long, narrow oysters are of little commercial value.

Oyster mariculture (Heffernan and Walker 1988) and stock transfer from overcrowded natural beds (Linton 1968) are two possible means of increasing the quality of oysters in Georgia. In order for either to be economically successful a field grow out system must be established which will eliminate or control the amount of spat settlement on oysters being cultured. The purpose of this study was to determine whether site of deployment and/or rotational regimens (inversion of growing bags seasonally, once monthly, or twice monthly) of oysters placed in protective growing bags could effectively eliminate oyster spat settlement in an oyster culture system.

TABLE 3.

Mean size of oysters placed in 13 mm mesh oyster growing bags (75/bag when deployed in Dec 1988) in Skidaway River, Georgia and rotated at various times. Subsets (N = 30/bag) were measured trimonthly. Mean shell length (SL) is given in mm  $\pm$  one standard error (SE).

Area/Monthly Rotation	Dec 1988	Mar 1989	Jun 1989		Sept 1989	
	S	S	S	N	S	N
<i>Intertidal Bottom</i>						
Seasonal						
1	41.9 $\pm$ 0.95	51.8 $\pm$ 1.49	52.6 $\pm$ 1.56	30	55.7 $\pm$ 1.23	30
2	42.5 $\pm$ 0.89	51.4 $\pm$ 1.55	54.2 $\pm$ 1.70	30	57.1 $\pm$ 1.47	30
3	42.0 $\pm$ 0.88	51.1 $\pm$ 1.39	56.1 $\pm$ 1.42	30	54.2 $\pm$ 1.34	30
Once						
1	42.3 $\pm$ 0.94	49.3 $\pm$ 1.13	52.6 $\pm$ 1.15	30	52.9 $\pm$ 1.19	30
2	41.9 $\pm$ 0.99	50.0 $\pm$ 1.63	52.3 $\pm$ 1.67	30	52.8 $\pm$ 1.45	30
3	42.0 $\pm$ 0.87	51.7 $\pm$ 1.67	52.8 $\pm$ 1.59	30	54.3 $\pm$ 1.50	30
Twice						
1	42.0 $\pm$ 1.13	47.8 $\pm$ 1.39	49.8 $\pm$ 1.87	30	54.0 $\pm$ 1.82	30
2	41.9 $\pm$ 1.25	52.2 $\pm$ 2.25	52.3 $\pm$ 1.96	30	53.3 $\pm$ 1.73	30
3	41.9 $\pm$ 0.95	52.2 $\pm$ 1.76	56.8 $\pm$ 1.63	30	53.8 $\pm$ 1.75	30
<i>Subtidal Bottom</i>						
Seasonal						
1	41.9 $\pm$ 1.12	54.8 $\pm$ 1.94	56.4 $\pm$ 2.22	30	60.7 $\pm$ 2.36	16
2	41.9 $\pm$ 0.74	50.7 $\pm$ 1.57	55.7 $\pm$ 1.18	30	56.4 $\pm$ 1.79	17
3	41.8 $\pm$ 1.01	51.6 $\pm$ 1.98	56.2 $\pm$ 1.83	30	58.9 $\pm$ 2.75	12
Once						
1	41.9 $\pm$ 1.04	52.5 $\pm$ 1.82	Lost		Lost	
2	42.0 $\pm$ 1.03	52.2 $\pm$ 1.45	Lost		Lost	
3	41.6 $\pm$ 0.99	50.3 $\pm$ 1.61	Lost		Lost	
Twice						
1	41.9 $\pm$ 0.87	53.6 $\pm$ 1.93	54.4 $\pm$ 1.64	30	59.42 $\pm$ 1.70	30
2	42.0 $\pm$ 1.16	51.4 $\pm$ 1.77	56.7 $\pm$ 1.97	24	59.93 $\pm$ 2.56	16
3	41.9 $\pm$ 1.05	50.1 $\pm$ 1.93	54.9 $\pm$ 1.89	30	57.54 $\pm$ 1.94	27
<i>Subtidal Off Bottom</i>						
Seasonal						
1	42.4 $\pm$ 1.00	55.5 $\pm$ 1.72	59.2 $\pm$ 1.78	30	62.8 $\pm$ 1.66	30
2	42.4 $\pm$ 1.09	54.3 $\pm$ 1.52	58.7 $\pm$ 1.25	30	60.4 $\pm$ 1.52	30
3	42.0 $\pm$ 0.84	60.7 $\pm$ 1.72	60.3 $\pm$ 1.45	30	68.0 $\pm$ 1.20	30
Twice						
1	41.8 $\pm$ 1.14	55.2 $\pm$ 1.90	59.6 $\pm$ 1.51	30	64.5 $\pm$ 1.37	30
2	42.0 $\pm$ 0.99	54.2 $\pm$ 1.47	60.9 $\pm$ 1.62	30	62.7 $\pm$ 1.37	30
3	42.0 $\pm$ 0.96	56.8 $\pm$ 1.72	58.6 $\pm$ 1.75	30	68.4 $\pm$ 1.88	30

S =  $\bar{x}$  SL  $\pm$  SE

N = No. oysters sampled

## METHODS

Juvenile oysters were collected underneath the University of Georgia Marine Extension Service and Skidaway Institute of Oceanography docks on Skidaway Island. Clumps of oysters were separated into single oysters and all spat were removed. Oysters with shell lengths (SL) of 30.0–70.0 mm were retained for this study. Shell length was measured (hinge to lip), oysters were placed into 13 mm mesh (50  $\times$  50 cm) oyster growing bags (n = 75 per bag) and deployed in the Skidaway River in December 1988. All replicates had a mean SL = 41.9  $\pm$  1 mm. Bags of oysters were placed intertidally on the river bottom, subtidally on the river bottom, and subtidally off-bottom and rotated (turned upside down) seasonally (trimonthly), once

monthly, or twice monthly (Table 1). Replicate bags (n = 3) of oysters were attached in a line to two PVC poles via cable ties for each treatment. On-bottom units were laid upon the intertidal (plus 2 hours above the mean low water mark where the oysters were exposed approximately 8 hours a day) or subtidal river bottom and anchored in place by two L-shaped aluminum poles positioned at the mid section of each unit. Subtidal off-bottom treatments were attached by cable ties to a trestle constructed of 3 mm reinforcement rods. Subtidal off-bottom units were approximately 0.3 m above the river bottom.

Random samples of oysters (n = 30) from each bag were examined for number of spat per oyster and measured for SL, using a Vernier caliper to the nearest 0.5 mm, in March, June, and September 1989. Total live counts per

TABLE 4.

Survival of oysters placed in 13 mm mesh oyster growing bags (75/ bag when deployed in Dec 1988) in Skidaway River and rotated at various times.

Area/Monthly Rotation	Mar 1989 % Survival	Jun 1989 % Survival	Sept 1989 % Survival
<i>Intertidal Bottom</i>			
Seasonal			
1	90.7	78.7	65.3
2	85.3	74.7	61.3
3	90.7	84.0	62.7
Once			
1	84.0	68.0	50.7
2	73.3	66.7	52.0
3	84.0	84.0	61.3
Twice			
1	77.3	65.3	50.7
2	76.0	65.3	48.0
3	85.3	76.0	65.3
<i>Subtidal Bottom</i>			
Seasonal			
1	57.3	54.7	21.3
2	60.0	54.7	22.7
3	56.0	45.3	16.0
Once			
1	72.0	Lost	Lost
2	52.0	Lost	Lost
3	57.3	Lost	Lost
Twice			
1	70.7	68.0	41.3
2	41.3	32.0	21.3
3	60.0	56.0	36.0
<i>Subtidal Off Bottom</i>			
Seasonal			
1	82.7	78.7	65.3
2	84.0	66.7	56.0
3	96.0	92.0	78.7
Twice			
1	90.7	76.0	61.3
2	85.3	82.7	72.0
3	86.7	84.0	74.6

TABLE 5B.

Results of the Tukey multiple range tests ( $P < 0.05$ ; mean number of spat/oyster by area) for oysters which were placed in 13 mm mesh oyster growing bags in Skidaway River, Georgia and rotated at various times.

<i>June 1989</i>			
Area	Inter	Sub	Sub
	Bot	Bot	Off-Bot
$\bar{x}$ No. Spat	1.1	1.2	26.3
<i>September 1989</i>			
Area	Sub	Inter	Sub
	Bot	Bot	Off-Bot
$\bar{x}$ No. Spat	0.2	0.9	26.7

Sub = Subtidal

Inter = Intertidal

Bot = Bags were placed directly on the river bottom

Off-Bot = Bags were placed off the river bottom on trestles

bag were also recorded. The subtidal bottom monthly rotated treatment was lost between the March and June sampling dates.

Mean values for number of spat per oyster (Table 2) as well as SL (Table 3) were computed for each replicate on all sampling dates. Percent survival was also determined (Table 4). Statistical analysis of number of spat per oyster and SL were evaluated using a nested Analysis of Variance (ANOVA) and Tukey's multiple range test ( $P < 0.05$ ). Percent survival was arcsine transformed and evaluated using a nested ANOVA and Tukey's multiple range test. All ANOVA's and multiple range tests were carried out using SAS/PC software. The SAS program was unable to compute F values because the data was unbalanced (number of treatments in each area were not equal), but it did compute the mean squares (MS). The F values were obtained by dividing the area MS by treatment MS, treatment MS by replicate MS, and replicate MS by within

TABLE 5A.

Results of the nested Analysis of Variance (ANOVA) tests ( $P < 0.05$ ) for the mean number of oyster spat settled on oysters which were placed in 13 mm mesh oyster growing bags in Skidaway River, Georgia and rotated at various times.

Factor	df	Mean Squares	F Ratio	F Prob
<i>June 1989</i>				
Nested ANOVA				
Area	2	40241.000	569.147	<0.001
Treatment	4	70.704	0.455	NS
Replicate	14	155.235	3.379	<0.001
Within Groups	603	45.936		
<i>September 1989</i>				
Nested ANOVA				
Area	2	41272.000	1030.178	<0.001
Treatment	4	40.063	0.234	NS
Replicate	14	171.387	3.406	<0.001
Within Groups	545	50.313		

TABLE 6A.

Results of the nested Analysis of Variance (ANOVA) tests ( $P < 0.05$ ) for mean oyster shell length (SL in mm) when placed in 13 mm mesh oyster growing bags and rotated at various times.

Factor	df	Mean Squares	F Ratio	F Prob
<i>December 1988</i>				
Nested ANOVA				
Area	2	849.406	2.901	NS
Treatment	5	406.151	1.768	NS
Replicate	16	229.661	0.031	NS
Within Groups	1776	7516.684		
<i>March 1989</i>				
Nested ANOVA				
Area	2	159971.000	47.584	<0.001
Treatment	5	3361.838	0.294	NS
Replicate	16	11433.000	1.324	NS
Within Groups	696	8634.427		
<i>June 1989</i>				
Nested ANOVA				
Area	2	212725.000	44.801	0.001 < p < 0.005
Treatment	4	4748.195	0.585	NS
Replicate	14	8110.543	0.981	NS
Within Groups	603	8264.355		
<i>September 1989</i>				
Nested ANOVA				
Area	2	556109.000	54.170	0.001 < p < 0.005
Treatment	4	10266.000	0.810	NS
Replicate	14	12670.000	1.721	NS
Within Groups	538	7363.706		

group MS. The probability associated with each F value was then obtained from Rohlf and Sokal (1981).

### RESULTS

Rotation was found to have no effect on mean number of spat settled on each oyster, or growth and survival rates of

TABLE 6B.

Results of the Tukey multiple range tests ( $P < 0.05$ ; mean shell length by area) for oysters which were placed in 13 mm mesh oyster growing bags in Skidaway River, Georgia and rotated at various times. Shell length (SL) in mm.

<i>March 1989</i>			
Area	Inter	Sub	Sub
	Bot	Bot	Off-Bot
$\bar{x}$ SL (mm)	50.8	51.9	56.1
<i>June 1989</i>			
Area	Inter	Sub	Sub
	Bot	Bot	Off-Bot
$\bar{x}$ SL (mm)	53.3	55.7	59.6
<i>September 1989</i>			
Area	Inter	Sub	Sub
	Bot	Bot	Off-Bot
$\bar{x}$ SL (mm)	54.2	58.8	64.5

Sub = Subtidal

Inter = Intertidal

Bot = Bags were placed directly on the river bottom

Off-Bot = Bags were placed off the river bottom on trestles

oysters for each of the individual deployment sites (Tables 5a, 6a, 7a); however, significant differences were found within each of these parameters based on area of deployment.

Oyster spat settlement (Tables 2, 5a,b) was significantly greater on oysters grown subtidally off-bottom (Sept,  $\bar{x}$  = 26.7 spat/oyster) than on oysters cultured on the subtidal bottom (Sept,  $\bar{x}$  = 0.2 spat/oyster) or the intertidal bottom (Sept,  $\bar{x}$  = 0.9 spat/oyster). Subtidal off-bottom oysters collected 133 times more spat than did the subtidal bottom oysters. During the September sampling, some of the subtidal off-bottom oysters were so heavily covered with spat that the original test oysters were difficult to distinguish. The nested ANOVA for mean number of spat (Table 5a) also shows significant differences among replicates which are probably due to the uneven distribution of variance within the replicates of some treatments (Table 2).

Mean SL was found to be significantly greater for oysters deployed subtidally off-bottom (Tables 3, 6a,b) which had a mean increase in SL of 22.4 mm (Dec 1988,  $\bar{x}$  SL–Sept 1989,  $\bar{x}$  SL). Subtidal bottom oysters exhibited a larger mean increase in SL ( $\bar{x}$  = 16.9 mm) than intertidal bottom oysters ( $\bar{x}$  = 12.2 mm).

Significant differences in arcsine transformed survival data were evident from a nested ANOVA (Tables 4, 7a). Tukey's multiple range test (Table 7b) showed that oysters placed subtidally off-bottom (Sept,  $\bar{x}$  % survival = 68.0) exhibited the greatest amount of survival. The intertidal

TABLE 7A.

Results of the nested Analysis of Variance (ANOVA) tests ( $P < 0.05$ ) for arcsine transformed survival data of oysters which were placed in 13 mm mesh oyster growing bags in Skidaway River, Georgia and rotated at various times.

Factor	df	Mean Squares	F Ratio	F Prob
<i>March 1989</i>				
Nested ANOVA				
Area	2	0.449	37.417	<0.001
Treatment	5	0.112	0.923	NS
Within Groups	16	0.013		
<i>June 1989</i>				
Nested ANOVA				
Area	2	0.249	27.667	0.001 < p < 0.005
Treatment	4	0.009	0.450	NS
Within Groups	14	0.020		
<i>September 1989</i>				
Nested ANOVA				
Area	2	0.379	31.583	0.001 < p < 0.005
Treatment	4	0.012	1.333	NS
Within Groups	14	0.009		

bottom area (Sept,  $\bar{x}$  % survival = 57.5) had higher survival rates (Table 4) than the subtidal bottom area (Sept,  $\bar{x}$  % survival = 26.4). The subtidal bottom oysters suffered particularly high mortalities between June and September (Tables 4, 7a) during the height of the spawning season when this area exhibited 25% mortality (Tables 4, 7b).

#### DISCUSSION

This study was designed to determine the feasibility of eliminating or controlling oyster spat settlement in the field by oyster bag rotation and/or site of deployment of oysters cultured within bags. Although rotation had no effects, the results herein clearly show that deployment of oyster bags

on both the intertidal and subtidal bottoms produced significantly lower spatfall rates on the cultured oysters than those placed subtidally off-bottom. The reason for this difference in spat settlement is unknown. In a study of estuarine epibenthic fouling on oyster shells (Rheinhardt and Mann 1990), it was found that 42–59% of the shell coverage became fouled with sediment and detritus. It is postulated that the accumulation of these materials on oysters placed on the intertidal and subtidal bottoms in this study may discourage spat settlement.

Oysters have been shown to grow faster in off-bottom as opposed to on bottom culture systems (Matthiesen and Toner 1966, Shaw 1968); however, in this study oysters grown off-bottom became heavily covered with oyster spat effectively eliminating this zone as a culture site during the oyster spawning season. Therefore, sacrificing some growth during the spawning season by placement of oysters on the bottom to avoid fouling by spat is essential in order to retain a higher market value product.

In terms of the intensity of spatfall, intertidal and subtidal bottom oysters did not differ; however, differences occurred between these two deployment sites in terms of growth (SL) and survival rates. These differences may be due in part to reduced exposure and greater feeding opportunities by the subtidal oysters. Exposure of intertidal oysters to daily maximum temperatures  $>32^{\circ}\text{C}$  for 73 days from April through August 1989 and daily minimum temperatures of  $<0^{\circ}\text{C}$  for 19 days from December 1988 through February 1989 (U.S. Dept. of Commerce 1988, 1989) might have contributed to lower growth (SL) rates of oysters in the intertidal area. Oysters placed intertidally on the bottom grew less during the spawning season than did oysters deployed subtidally (Tables 3, 6b), probably due to combined stress of exposure during low tide and spawning. Subtidal bottom oysters which were rotated twice monthly

TABLE 7B.

Results of the Tukey multiple range tests ( $P < 0.05$ ) for percent survival data of oysters placed in 13 mm mesh oyster growing bags in Skidaway River, Georgia and rotated at various times.

<i>March 1989</i>			
Area/	Sub	Inter	Sub
	Bot	Bot	Off-Bot
% Survival	58.5	83.0	87.6
<i>June 1989</i>			
Area/	Sub	Inter	Sub
	Bot	Bot	Off-Bot
% Survival	51.8	73.6	80.0
<i>September 1989</i>			
Area/	Sub	Inter	Sub
	Bot	Bot	Off-Bot
% Survival	26.4	57.5	68.0

Inter = Intertidal

Sub = Subtidal

Bot = Bags were placed directly on the river bottom

Off-Bot = Bags were placed on trestles in the river

Seas = Seasonal

had a larger increase in SL during the period encompassing the spawning season (June to Sept) than in the previous period from March to June (Tables 3, 6b); however this area had the lowest survival rates throughout the study, possibly due to predation losses. Oysters grown intertidally on bottom were exposed during low tides and therefore were less accessible to predators during those times. The oysters cultured subtidally off-bottom were placed on trestles approximately 0.3 m off the river bottom which may have deterred predation. *Perkinsus marinus* (Dermo) could also have been a factor causing decreased survival rates; however, it is expected that the combination of disease and physiological stress on oysters in the intertidal bottom area would have caused greater mortality than disease alone in either of the subtidal areas.

Further study comparing growth and survival rates of oysters deployed on intertidal versus subtidal bottoms exclusively during the spawning season might delineate the advantages and disadvantages of the two areas. Furthermore, studies of growth and survival of oysters planted at various intertidal heights may reveal an optimum area of oyster deployment in terms of both growth and survival.

#### MARICULTURE RECOMMENDATIONS

An important finding of this study is that oyster spat settlement can be managed by oyster bag deployment. This overcomes one of the major problems which had prohibited oyster mariculture to date. It creates the possibility of producing single oysters which would represent an important commercial commodity in comparison to currently available natural oyster stocks. Cultivation of oysters in bags should be carried out subtidally off-bottom in Georgia during the non-spawning season (Nov–May) in order to maintain maximum growth rates. Further studies on settlement dynamics may lengthen this period. Oysters should also be transferred to an intertidal bottom site during the spawning season to eliminate fouling by oyster spat.

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## FUNCTIONAL SEROTONIN RECEPTOR SITES ON ATLANTIC SURFCLAM *SPISULA SOLIDISSIMA* (DILLWYN, 1817) OOCYTES AND SPERM

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**ABSTRACT** The 5-HT receptor site-selective agonists, 8-OH-DPAT (5-HT<sub>1A</sub>), and  $\alpha$ -methyl-5-HT (5-HT<sub>2</sub>) induced maturation of *Spisula* oocytes *in vitro*. The 5-HT antagonists, mianserin (5-HT<sub>1</sub>, 5-HT<sub>2</sub>) and ketanserin (5-HT<sub>2</sub>) were the most effective inhibitors of 5-HT-induced GVBD in *Spisula* oocytes. Mianserin also blocked oocyte maturation induced with 8-OH-DPAT and  $\alpha$ -methyl-5-HT. Ketanserin inhibited maturation induced with  $\alpha$ -methyl-5-HT but not with 8-OH-DPAT. BMY 7378 (5-HT<sub>1A</sub>), on the other hand, inhibited maturation induced with 8-OH-DPAT but not with  $\alpha$ -methyl-5-HT. The present results suggest that *Spisula* oocytes possess 5-HT receptors sensitive to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> site-selective analogs.

The 5-HT agonists, 8-OH-DPAT,  $\alpha$ -methyl-5-HT and 2-methyl-5-HT, stimulated the motility of cold-immobilized *Spisula* sperm. The 5-HT antagonists, mianserin, ketanserin, ICS 250-930 and GR 38032F, blocked *Spisula* sperm motility stimulated with 5-HT, 8-OH-DPAT and  $\alpha$ -methyl-5-HT. The present results demonstrate that the 5-HT receptor of *Spisula* sperm is sensitive to 5-HT<sub>1A</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> site-selective analogs. We hypothesize that the 5-HT receptor associated with *Spisula* gametes may be a complex structure composed of multiple ligand binding sites.

**KEY WORDS:** sperm motility, oocyte maturation, serotonin, 5-hydroxytryptamine, receptor sites, neurotransmitter

### INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) induces spawning of gametes when injected directly into the gonads of bivalve taxa, including *Patinopecten yessoensis*, *Tridacna*, and *Spisula solidissima* (Matsutani and Nomura 1982, Gibbons and Castagna 1984, Braley 1985, Hirai et al. 1988). 5-HT triggers *in vitro* maturation of bivalve oocytes, including *Crassostrea gigas*, *Tapes*, *Philippinarum* and *S. solidissima* (Osanai and Kuraishi 1988, Hirai et al. 1988). The physiological relevance of these findings is supported by the identification of 5-HT in the body fluid and ganglion cells of the surf clam (Welsh and Moorhead 1960, Kadam and Koide 1989a).

Immunoreactivity for 5-HT was localized to a large number of perikarya of the cerebral, pedal and accessory ganglia of the scallop (Matsutani and Nomura 1986). In the gonadal region, immunoreactive fibers were located around the gonoduct and along the germinal epithelium, indicating that 5-HT plays a role in spawning. In previous studies, we have demonstrated that 8-OH-DPAT, a 5-HT<sub>1A</sub> receptor site agonist (Middlemiss and Fozard 1983), induces oocyte

maturation (Kadam and Koide 1989b) and 5-HT stimulates the motility of cold-immobilized *Spisula* sperm (Kadam and Koide 1990).

In the present study, receptor site-selective 5-HT agonists and antagonists (Bradley et al. 1986, Göthert 1986, Petroutko 1988) were used to characterize the 5-HT receptor types on *Spisula* oocytes and sperm. Data are presented showing that the receptor on *Spisula* oocytes and sperm is sensitive to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> site-selecting analogs, and in addition, that the sperm receptor is activated by 5-HT<sub>3</sub> analogs.

### MATERIALS AND METHODS

#### Chemicals

PAPP (LY-165, 163), 8-OH-DPAT,  $\alpha$ -methyl-5-HT, CGS 12066B dimaleate,  $\alpha$ -methyl-5-HT, quipazine maleate, ICS-205-930, ketanserin, MDL 7222, mianserin, (-)-propranolol hydrochloride and 5-HT creatinine sulfate were purchased from Research Biochemicals, Inc. The following compounds were obtained as gifts: methysergide maleate from Sandoz Pharmaceutical; GR 38032F from Glaxo; and BMY 7378 from Bristol-Myers.

#### Assay for Oocyte Maturation

Female surf clams were obtained from the Marine Resources Dept., Marine Biological Laboratory, Woods

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Hole, Massachusetts. The ovaries were dissected, placed in artificial sea water (ASW), cut into small pieces with a pair of scissors and the freed oocytes collected by sedimentation at unit gravity. Oocytes were washed several times by settling. Stock solutions of the agonists and antagonists were prepared according to their solubility in ASW or ethanol and were added to a suspension of oocytes ( $2000 \text{ ml}^{-1}$ ) at varying final concentrations ranging from 1 to  $50 \mu\text{M}$ . In testing the antagonists the oocytes were incubated with each compound for 10 min, and maturation was then induced with 5-HT at a concentration of  $5 \mu\text{M}$ . The oocytes were examined by light microscopy 15 and 30 min after the addition of 5-HT, for the presence or absence of a germinal vesicle (GV). The incidence of spontaneous maturation among the isolated oocytes was less than 5%.

#### Collection of Sperm

Testes were excised from male surf clams and the milky semen collected with a Pasteur pipet. The semen was diluted 1:500 with ASW. The sperm suspension was centrifuged at 300g for 5 min to remove debris. The supernatant was centrifuged at 1000g for 10 min at  $4^\circ\text{C}$ . The pellet containing sperm was suspended in ASW equivalent to the original volume. The sperm suspension was stored over-

night at  $4^\circ\text{C}$  and added to ASW at ambient temperature ( $20\text{--}22^\circ\text{C}$ ) before use in the motility assay. Over 98% of the sperm remained immotile for 30 min.

#### Sperm Motility Assay

Cold-immobilized sperm were dispensed by adding 50  $\mu\text{l}$ , or one drop to one ml of ASW at  $20\text{--}22^\circ\text{C}$ . 5-HT or an analog was added to make final concentrations ranging from 1 to  $50 \mu\text{M}$ . Stimulation of sperm motility could be detected within 1 min by examination with a light microscope. Motility increase occurred in over 95% of sperm; the response was striking and practically instantaneous.

#### Assay for Inhibition of Sperm Motility

To one ml of *Spisula* sperm suspension at  $20\text{--}22^\circ\text{C}$ , 5-HT antagonists were added to make the appropriate final concentration, and the suspension was incubated at  $20\text{--}22^\circ\text{C}$  for 5 min. 5-HT and other agonists, dissolved in

TABLE 1.

Induction of maturation of *Spisula* oocytes with 5-HT agonists.

Compounds <sup>1</sup>	Receptor Selective Type	Concentration ( $\mu\text{M}$ )	GVBD (%)		
8-OH-DPAT	5-HT <sub>1A</sub>	50	100		
		25	100		
		10	100		
		5	98		
		2	95		
$\alpha$ -methyl-5-HT	5-HT <sub>2</sub>	50	100		
		25	100		
		10	100		
		5	91.3		
PAPP	5-HT <sub>1A</sub>	50	0		
		m-CPP-HCl	5HT <sub>1</sub> , 5-HT <sub>2</sub>	50	0
		5-CT	5-HT <sub>1</sub>	50	0
CGS 12066B	5-HT <sub>1B</sub>	50	0		
1-phenyl biguanide	5-HT <sub>3</sub>	50	0		
2-methyl-5-HT	5-HT <sub>3</sub>	50	0		

<sup>1</sup> Abbreviations: PAPP (LY-165, 163), p-aminophenylethyl-m-trifluoromethyl-phenyl piperazine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)-tetralin;  $\alpha$ -methyl-5-HT,  $\alpha$ -methyl-5-hydroxytryptamine maleate; m-CPP-HCl, 1-(3-chlorophenyl) piperazine dihydrochloride; 5-CT, 5-carboxamidotryptamine maleate, 3-(2-aminoethyl)-1H-indole-5-carboxamide maleate; ICS 205-930, 3-tropanyl-indole-3-carboxylate; MDL 7222, 3-tropanyl-3,5-dichlorobenzoate; quipazine dimaleate, 2-(1-piperazinyl) quinoline dimaleate; GR38032F, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl-4H-carbazol-4-one] hydrochloride dihydrate; CGS-12066B, 7-trifluoromethyl-4(4-methyl-1-piperazinyl) pyrrolo[1,2- $\alpha$ ]quinoxaline, 1:2 maleate; phenyl biguanide, N-phenylimidodicarbonimidic diamide; and 5-HT, serotonin, 5-hydroxytryptamine.

TABLE 2.

Inhibition of 5-HT-induced maturation of *Spisula* oocytes by 5-HT antagonists.

Compounds <sup>1</sup>	Receptor Selective Type	Concentration ( $\mu\text{M}$ )	Inhibition of GVBD (%)
mianserin	5-HT <sub>1</sub> , 5-HT <sub>2</sub>	50	93.7
		25	90.6
		10	90.3
		5	87.3
		2	77.8
ketanserin	5-HT <sub>2</sub>	1	70.7
		50	95.4
		25	92.3
		10	88.0
		5	83.0
GR 38320F	5-HT <sub>3</sub>	2	80.8
		50	94.6
		25	91.6
		10	89.7
		5	67.3
BMY 7378	5-HT <sub>1A</sub>	50	96.9
		25	90.3
		10	57.4
methysergide	5-HT <sub>1</sub>	5	10.5
		50	97.3
		25	96.6
quipazine dimaleate	5-HT <sub>3</sub> , 5-HT <sub>2</sub> , 5-HT <sub>1</sub>	10	70.4
		5	3.5
		50	94.4
		25	82.0
		10	0
(–)-propranolol HCl	5-HT <sub>1</sub>	50	99.4
		25	90.5
		10	80.6
MDL 7222	5-HT <sub>3</sub>	5	76.9
		50	5.0
ICS 205-930	5-HT <sub>3</sub>	50	7.6

<sup>1</sup> Abbreviations are given in Table 1.

ASW, were added to make a final concentration of 5 μM. Percent sperm motility was assessed immediately, as described above.

**RESULTS**

Receptor site-selective 5-HT agonists were assayed for oocyte maturation-inducing activity. 8-OH-DPAT (5-HT<sub>1A</sub>) and α-methyl-5-HT (5-HT<sub>2</sub>) were the only active compounds among eight agonists tested (Table 1). They were effective at 2 μM. The agonists, PAPP (5-HT<sub>1A</sub>) and 5-CT (5-HT<sub>1</sub>), were inactive at concentrations as high as 50 μM.

Several receptor site-selective 5-HT antagonists were tested for their capacity to block 5-HT-induced maturation (Table 2). Mianserin (5-HT<sub>1</sub>, 5-HT<sub>2</sub>) and ketanserin (5-HT<sub>2</sub>) were the most effective inhibitors. To validate that *Spisula* oocytes are responsive to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptor site-selective compounds, 8-OH-DPAT and α-methyl-5-HT were used to induce dissolution of the GV, designated as GV breakdown (GVBD) and the corresponding receptor selective antagonists were administered to block their actions. BMY 7378 (5-HT<sub>1A</sub>) inhibited 8-OH-DPAT-induced maturation, but did not influence the action of α-methyl-5-HT (5-HT<sub>2</sub>), and mianserin (5-HT<sub>1</sub> and 5-HT<sub>2</sub>) blocked the inducing activity of both agonists (Table 3). Ketanserin (5-HT<sub>2</sub>) effectively blocked only α-methyl-5-HT (5-HT<sub>2</sub>) induction.

Various 5-HT receptor site-selective agonists and antagonists were assayed for their capacity to stimulate and to block motility of cold-immobilized *Spisula* sperm, respectively (Tables 4, 5). The most effective agonists were 8-OH-DPAT (5-HT<sub>1A</sub>) and α-methyl-5-HT (5-HT<sub>2</sub>). They were effective at 1 μM. 5-CT (5-HT<sub>1</sub>) was active at a higher concentration (50 μM), and 2-methyl-5-HT (5-HT<sub>3</sub>) at 50 μM induced slight stimulation. On the other hand, PAPP (5-HT<sub>1A</sub>), CGS-12066β (5-HT<sub>1B</sub>), m-CPP-HCl (5-HT<sub>1</sub>, 5-HT<sub>2</sub>), and 1-phenyl biguanide (5-HT<sub>3</sub>) were inactive. The most effective blocker was mianserin (5-HT<sub>1</sub>, 5-HT<sub>2</sub>). The 5-HT<sub>3</sub> receptor site-selective antagonists, ICS 205-930 and GR 38032F, were effective blockers at higher concentration (50 μM), whereas the 5-HT<sub>2</sub> antagonist, ketanserin, showed moderate inhibitory activity. On the other

**TABLE 4.**

Stimulation of *Spisula* sperm motility by 5-HT agonists.

Compounds	Receptor Site Selective Type	Concentration† (μM)	Stimulation of Motility (%)
8-OH-DPAT	5-HT <sub>1A</sub>	1	100
α-methyl-5-HT	5-HT <sub>2</sub>	1	100
5-CT	5-HT <sub>1</sub>	50	100
2-methyl-5-HT	5-HT <sub>3</sub>	50	25
PAPP	5-HT <sub>1A</sub>	50	0
CGS-12066β dimaleate	5-HT <sub>1B</sub>	50	0
m-CPP-HCl	5-HT, 5-HT <sub>2</sub>	50	0
1-phenyl biguanide	5-HT <sub>3</sub>	50	0

hand, quipazine (5-HT<sub>3</sub>), methysergide (5-HT<sub>1</sub>) and BMY-7378 (5-HT<sub>1A</sub>) were inactive.

To validate that *Spisula* sperm possess 5-HT receptors responsive to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptor site-selective analogs, the complementary antagonists were used to block 8-OH-DPAT and α-methyl-5-HT-stimulated sperm motility. Mianserin (5-HT<sub>1</sub>, 5-HT<sub>2</sub>) at 25 μM blocked the motility-stimulating activity of both agonists. An unexpected finding was that the 5-HT<sub>3</sub> receptor site-selective antagonists, ICS-205-930 and GR 38032F (50 μM), blocked 8-OH-DPAT-stimulated motility by 25%, whereas BMY 7378 (5-HT<sub>1A</sub>) was inactive.

**DISCUSSION**

The 5-HT receptor of *Spisula* oocytes is sensitive to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> site-selective analogs. This contention is supported by the maturation inducing activity of the receptor site-selective agonists, 8-OH-DPAT (5-HT<sub>1A</sub>) and α-methyl-5-HT (5-HT<sub>2</sub>), and its blockade by mianserin (5-HT<sub>1</sub>, 5-HT<sub>2</sub>) and ketanserin (5-HT<sub>2</sub>) (Leysen et al. 1982, Middlemiss and Fozard 1983). In addition, BMY 7378 (5-HT<sub>1A</sub> antagonist) inhibited the maturation-inducing activity of 8-OH-DPAT but not that of α-methyl-5-HT. The inactivity of the piperazine agonists, PAPP (5-HT<sub>1A</sub>), and m-CPP-HCl (5-HT<sub>1</sub>, 5-HT<sub>2</sub>), suggests that the 5-HT receptor of *Spisula* oocytes possesses structural specificity (Glennon 1987). The inability of 5-CT (5-HT<sub>1</sub>) to induce

**TABLE 3.**

Induction of *Spisula* oocyte maturation by 5-HT receptor site-selective agonists and inhibition by site-selective antagonists.

5-HT Agonists	Receptor Selective Type	5-HT Antagonists	Receptor Selective Type	Inhibition (%)
8-OH-DPAT	5-HT <sub>1A</sub>	BMY 7378	5-HT <sub>1A</sub>	88
8-OH-DPAT	5-HT <sub>1A</sub>	mianserin	5-HT <sub>1</sub> , 5-HT <sub>2</sub>	100
α-methyl-5-HT	5-HT <sub>2</sub>	mianserin	5-HT <sub>1</sub> , 5-HT <sub>2</sub>	100
α-methyl-5-HT	5-HT <sub>2</sub>	ketanserin	5-HT <sub>2</sub>	75
α-methyl-5-HT	5-HT <sub>2</sub>	BMY 7378	5-HT <sub>1A</sub>	0

TABLE 5.

Inhibition of 5-HT-induced stimulation of *Spisula* sperm motility by 5-HT antagonists.

Compounds	Receptor Site Selective Type	Concentration* ( $\mu$ M)	Inhibition (%)
mianserin	5-HT <sub>1</sub> , 5-HT <sub>2</sub>	25	80
ICS 205-930	5-HT <sub>3</sub>	50	90
GR 38032F	5-HT <sub>3</sub>	50	90
ketanserin	5-HT <sub>2</sub>	50	50
quipazine maleate	5-HT <sub>3</sub>	50	0
methysergide	5-HT <sub>1</sub>	50	0
(-)-propranolol HCl	$\beta$ -adrenergic blocker 5-HT <sub>1</sub>	50	0
spiperone HCl	Dopamine D receptor	50	0
BMY 7378	5-HT <sub>1A</sub>	50	0

GVBD is revealing because this analog is an effective agonist and increases intracellular cAMP level of smooth muscle cells of the vena cava (Trevethick et al. 1986). Perhaps 5-CT may increase the oocyte cAMP level, thereby sustaining the meiotic arrest rather than triggering GVBD (Sato and Koide 1987). Also, the present findings suggest that the 5-HT receptors in *Spisula* oocytes differ from those in mammalian neurons.

*Spisula* oocytes probably do not possess functional 5-HT<sub>3</sub> receptor sites since the 5-HT<sub>3</sub> receptor site-selective agonists, 2-methyl-5-HT and phenyl biguanide, were inactive, and since 5-HT-induced maturation was not blocked by MDL 7222 or ICS 205-930 (Fozard 1984, Richardson et al. 1985, Richardson and Engel 1986). The slight inhibition observed with quipazine and GR 38320F suggests cross-reaction with 5-HT<sub>1A</sub> or 5-HT<sub>2</sub> receptor site or both (Lansdown et al. 1980, Glennon 1987).

Since 8-OH-DPAT (5-HT<sub>1A</sub>) and  $\alpha$ -methyl-5-HT (5-HT<sub>2</sub>) stimulated the motility of cold-immobilized *Spisula* sperm, and mianserin (5-HT<sub>1</sub>, 5-HT<sub>2</sub>) blocked the stimulation, the functional receptor sites on *Spisula* sperm would seem to be 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> types (Petroutko and Snyder 1981, Leysen et al. 1982, Middlemiss and Fozard 1983, Bradley et al. 1986, Petroutko 1986). This contention is further supported by the abilities of 5-CT (5-HT<sub>1</sub>) to

stimulate sperm motility at high concentration and ketanserin (5-HT<sub>2</sub>) to block 5-HT-stimulated motility (Leysen et al. 1982, Trevethick et al. 1986).

A previous study has suggested that *Spisula* sperm possess functional 5-HT<sub>3</sub> receptor sites (Kadam and Koide 1990). The stimulation of sperm motility by the 5-HT<sub>3</sub> selective agonist, 2-methyl-5-HT, and its inhibition by the 5-HT<sub>3</sub> antagonists, ICS 205-903 and GR 38032F, support this notion (Fozard 1984, Bradley et al. 1986). The 5-HT<sub>3</sub> antagonist and agonist, quipazine and phenyl biguanide, respectively (Lansdown et al. 1980), were inactive, but none of the piperazine derivatives, (e.g., PAPP, CGS 2066 $\beta$ , quipazine, and m-CPP-HCl) or the non-indole compound (1-phenyl biguanide) affected sperm motility. Therefore, the 5-HT receptor of *Spisula* sperm must have structural constraint for specific chemical groups, (i.e., for indole and tetralin), but not for the piperazine moiety.

Although, in general, the same 5-HT site-selective analogs were active on both types of *Spisula* gametes, there were clear differences in their actions. 2-methyl-5-HT and 5-CT could stimulate sperm motility, but did not induce oocyte maturation (Kadam and Koide 1990). BMY-7873 (5-HT<sub>1A</sub>) inhibited 5-HT-induced oocyte maturation but did not block 5-HT stimulation of sperm motility. We conclude that the 5-HT receptors of *Spisula* oocytes and sperm are sensitive to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> site-selective analogs, whereas that of *Spisula* sperm is responsive to 5-HT<sub>3</sub> analogs.

The 5-HT receptor on *Spisula* oocytes and sperm may consist of multiple distinctly different receptors or it could be a single receptor with different binding sites. We prefer the latter model that 5-HT receptor of *Spisula* gametes is a complex structure composed of multiple 5-HT binding sites. In the course of evolution and cellular specialization, clones of neuronal cells may have undergone a selective process resulting in the retention of specific receptor sites. To determine whether *Spisula* gametes possess a single receptor or multiple 5-HT receptors, the gene encoding the receptor must be identified and characterized.

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## GAMETOGENIC CYCLE OF SEA SCALLOPS (*PLACOPECTEN MAGELLANICUS* (GMELIN, 1791)) IN THE MID-ATLANTIC BIGHT\*

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**ABSTRACT** Gametogenesis of the sea scallop, *Placopecten magellanicus* (Gmelin), from three areas within the mid-Atlantic Bight was examined from January to December 1988. Histological and morphometric quantification of gonadal tissue concluded that a semiannual gametogenic cycle was characteristic of sea scallops from the mid-Atlantic Bight. The majority of spawning occurred in May and November. Gonadal development in spring comprised a longer period of time and resulted in greater fecundity than in fall. Differences were found in the timing and magnitude of the semiannual gametogenic processes between sex, area, and water depth within the study area. Varying temperature patterns between the mid-Atlantic Bight and more northerly resource areas may be partially responsible for the observed difference in gametogenic cycles. Semiannual spawning has potential implications for management strategies which are currently based on the assumption of annual spawning and recruitment events.

**KEY WORDS:** gametogenic cycle, sea scallop, *Placopecten magellanicus*, gonad weight

### INTRODUCTION

The sea scallop, *Placopecten magellanicus* (Gmelin), supports a valuable commercial fishery throughout much of its entire distribution in the northwestern Atlantic, from the Strait of Belle Isle, Newfoundland, to Cape Hatteras, N.C. (MacKenzie et al. 1978). The United States sea scallop fishery had a catch of over 33,700,000 pounds valued in excess of \$132,000,000 in 1989 (National Marine Fishery Service). Landings from Georges Bank have historically accounted for most of the United States harvests. The importance of the mid-Atlantic sea scallop fishery to the United States total commercial harvest has increased since the mid 1960s. This is primarily a result of an expanded fishery with numerous successful year classes and concomittant increases in fishing effort (New England Fishery Management Council et al. 1982).

Regulation and management of the sea scallop fishery is based, in part, on knowledge of the reproductive cycle of the species and the presumption that there is one stock distributed over several resource areas. On Georges Bank and in the Gulf of Maine, sea scallops undergo an annual gametogenic cycle with a single spawning period in the fall (MacKenzie et al. 1978, Robinson et al. 1982). Sea scallop harvesting in the United States is currently regulated by a minimum shell size restriction for scallops that are landed whole and a maximum average meat count (number of adductor muscles per pound) for scallops which are shucked at sea. In addition, there is a single temporary seasonal ad-

justment in the size restrictions to compensate for adductor muscle weight changes attributed to spawning in the fall. The seasonal adjustment was based on biological data from the Georges Bank area and the assumption that a similar reproductive pattern occurred in all scallop resource areas.

In the mid-Atlantic Bight, however, scallops appear to spawn twice a year (DuPaul et al. 1989). Given the relationship between adductor muscle weight and gametogenesis (Barber and Blake 1981, Robinson et al. 1981), the existence of a semiannual spawning event in the mid-Atlantic region implies that alternative regulatory strategies for each resource area may need to be considered. A detailed examination of gametogenic processes in this region is thus important for the management and regulation of the sea scallop fishery and for improving the utilization of the resource by commercial fishermen. This study examines gametogenesis of scallops within the mid-Atlantic Bight.

### MATERIALS AND METHODS

Whole fresh sea scallops, *Placopecten magellanicus*, were obtained from commercial fishing vessels operating in the mid-Atlantic Bight from January through December, 1988. Sample locations ranged from south of Long Island (40°00'N 73°00'W) to north of Cape Hatteras (37°30'N 74°30'W). This includes a rectangular area of approximately 28,000 km<sup>2</sup> running northeast to southwest in water depths of 40 to 74 m. Three areas within this range were sampled on a monthly basis. Consistent spatial-temporal sampling was not possible because of the commercial nature of the samples. The three areas included a site off the Virginia coast (37°15'–37°45'N), a site off the Delaware-Maryland peninsula (38°00'–38°30'N) and a site off the

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New Jersey coast (39°00'–40°00'N). These three sites were believed to adequately represent distinct geographical areas and be consistent with the requirements of stratified random sampling (Zar 1984).

At the time of sample collection, the date, time, Loran C coordinates, water depth (m), and surface water temperature (C) were recorded. Four to fifteen samples were collected monthly. Each sample consisted of 100 unshucked scallops randomly selected from 1.5 to 3.0 bushels of shellstock. The adductor muscle and gonad were resected from each animal. Wet weight of the gonad, including the crystalline style and foot, was measured to the nearest 0.1 gram. The maximum distance between dorsal and ventral margins (shell height) was measured to the nearest millimeter using a standard fish measuring board. One subsample was selected each month from the Delaware-Maryland area for estimation of dry gonad weights. Ten gonads, with crystalline style and foot removed, were dried to a constant weight in a drying oven (90°C) and reweighed.

Four to six subsamples were selected each month for histological examination. Twelve gonads of each sex, when sex could be visually determined, were used from each subsample. Pieces of gonad tissue (1 to 2 cm<sup>2</sup>) were cut from the middle anterior of the gonad. The tissue was preserved in Davidson's fixative, dehydrated, embedded in paraffin, cut 6 μm thick, mounted on slides, and stained with Harris' hematoxylin and eosin.

Volume fractions were determined for mature, developing, and resorbing gametes and nonreproductive matter following the methods of Weibel et al. (1966) and MacDonald and Thompson (1986). Five random fields of gonad tissue were examined with a compound microscope (150×) equipped with an eyepiece reticle. The reticle had a 10 mm square grid containing 61 points of intersecting lines. Volume fractions were calculated as the percentage of points occupied by each structure for each gonad section. A total of 1,326 gonads were examined histologically. Results are presented by individual areas, depths, and sex so that differences in reproductive processes can be examined and related to environmental conditions.

Mature gamete volume fractions for the three sample areas and depths were statistically examined to evaluate gametogenic processes within the mid-Atlantic Bight. The shallowest and deepest samples which were collected each month from the Virginia area were selected for water depth analysis. Volume fractions of mature gametes were selected for testing since this entity represents the potentially viable sexual products. The nonparametric Wilcoxon paired sample test was used to examine differences. Extreme heteroscedasticity precluded use of parametric tests (Steel and Torrie 1960).

Temporal changes in gonad weight were examined as an alternative monitoring method of gametogenesis. Scallops were grouped into five shell height intervals: 85–89 mm (N = 1,438), 90–94 mm (N = 1,859), 100–104 mm (N =

1,908), and 110–114 mm (N = 1,365). Mean gonad wet weight for each size interval was determined for each month with data pooled over sex and sample area. Gonad dry weight was estimated to compare the use of wet versus dry gonad weights as indicators of gametogenesis. Estimates of gonad dry weight were calculated as the product of individual gonad wet weight and the mean percentage of gonad dry weight, as determined from the representative monthly sample.

Fecundity was estimated by calculating the weight of mature gametes released upon spawning following the methods of Langton et al. (1987). Gamete weight of individual female scallops was estimated by the following regression model:

$$\ln G = \ln \beta_0 + \beta_1 \ln S + \beta_2 S + e$$

where  $G$  = gamete weight,  $S$  = height, and  $e$  = the random error term, assuming  $N(0, \sigma^2)$ . The equation is well suited for estimating the relationship between gonad weight and shell height. It allows for maximum weights and inflection points. The equation was estimated by ordinary least squares for the months characterized by max-

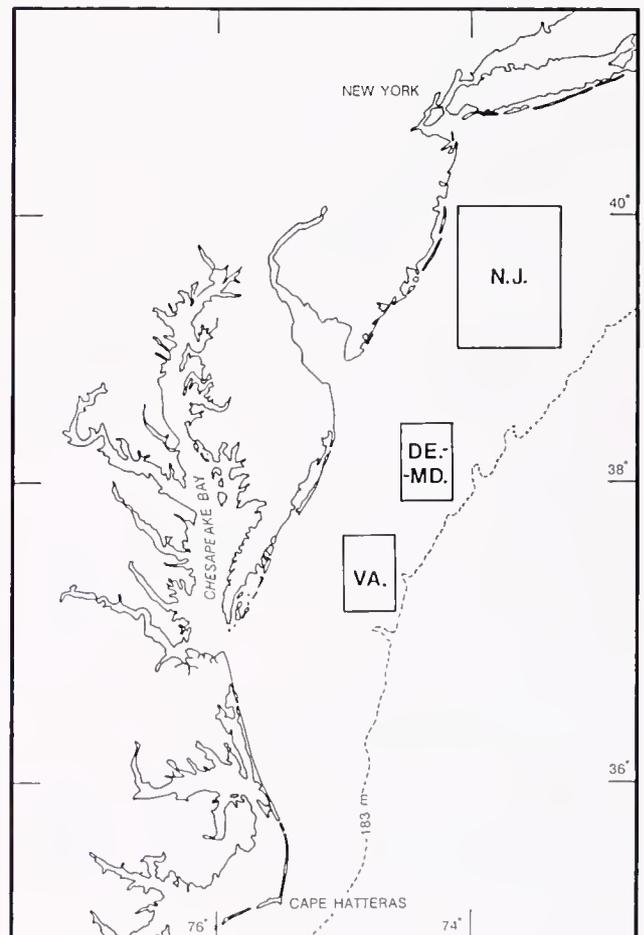


Figure 1. Location of Virginia (Va), Delaware-Maryland (De-Md), and New Jersey (Nj) sampling areas within the mid-Atlantic Bight.

imum and minimum values of the spawning cycle. Estimated weight loss was converted into ova numbers by dividing gamete wet weight loss by  $1.6 \times 10^{-7}$  g, the weight previously determined for wet mature ova (Langton et al. 1987).

Discrete bottom temperature data for the areas and months sampled during this study were not available. Bottom temperatures were therefore estimated with a computer program available from the Northeast Fisheries Center, National Marine Fisheries Service (NMFS) (Mountain 1989). The mean latitude and longitude coordinates from samples collected within the three areas were used to estimate temperatures. Coordinates were 37°31'N, 74°42'W (Virginia); 38°15'N, 74°11'W (Delaware-Maryland); 39°25'N, 73°08'W (New Jersey).

### RESULTS

During the study period, sea scallops from the mid-Atlantic Bight exhibited semiannual gametogenic cycles (Fig. 1). Within each sampling area, the majority of spawning of male and female scallops occurred during the same months. In spring, however, female scallops from the Delaware-Maryland area initiated spawning one month earlier than scallops from the New Jersey and Virginia areas, and were more completely spent by June. Unlike females, the percentage of mature gametes for males during this period showed little variation between areas prior to spring spawning. From April to August, mature gamete volume fractions in male and female gonads from Virginia and New Jersey areas gradually declined to minimal values in August. However, both sexes of Delaware-Maryland scallops exhibited a small increase in mature gamete volume fractions during July. During the fall gametogenic cycle, development and spawning occurred in a shorter time period and in a more simultaneous manner than in the spring. It was not possible to verify spawning for New Jersey scallops in the fall due to the lack of samples from that area in November, when spawning was likely to have occurred.

While the timing of gametogenic processes within each sampling area was very similar for both sexes, significant differences in mature gamete volume fractions were detected between sex in all three areas (Wilcoxon paired sample test: Virginia,  $z = -7.3338$ ,  $p < 0.0001$ ; Delaware-Maryland,  $z = -3.9653$ ,  $p < 0.0001$ ; New Jersey,  $z = -3.8760$ ,  $p < 0.0001$ ). Male scallops generally had larger volume fractions of mature gametes when ripe than females. Statistically significant differences were detected between mature gamete volume fractions for female scallops from the Delaware-Maryland and Virginia areas (Wilcoxon paired sample test,  $z = -4.5301$ ,  $p < 0.0001$ ). Significant differences were not detected in male scallops from the two sampling areas (Wilcoxon paired sample test,  $z = -1.7152$ ,  $p < 0.0863$ ).

Resorbed gametes comprised a small portion of the

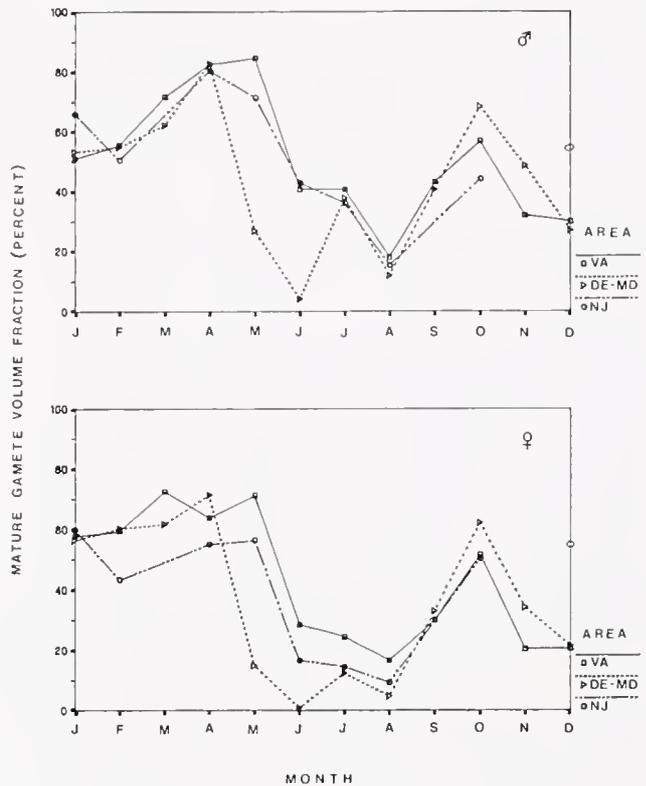


Figure 2. Monthly mean mature gamete volume fractions (percent) for male and female sea scallops from the Virginia (Va), Delaware-Maryland (De-Md), and New Jersey (NJ) sampling areas.

volume fractions in female gonads throughout the year, ranging from 0 to 15 percent. Seasonal changes in resorbed gamete volume fractions were similar to seasonal changes in mature gamete volume fractions, with the larger values occurring in the month of or prior to maximum development. The distribution and extent of lysis activity and resorption of oocytes within individual gonads was extremely variable. Initially, lysis was detectable by the breakdown of the vitelline membrane, which caused the oocytes to lose their round or polygonal shape and take on an irregular, jigsaw appearance. As lysis progressed and egg material was resorbed, empty space and fragments of oocytes were observed in the follicles. The occurrence of resorbed gametes reduced the percentage of mature gametes present in female gonads.

Results similar to those derived from histological quantification were obtained using wet and dry gonad weights as a measure of gametogenesis (Fig. 2). It was also evident that the pattern of gametogenesis did not differ significantly with shell size/age of scallops. Scallops 85–89 mm in shell height were sexually active. The percent of tissue remaining after removal of the foot and drying of the gonad varied seasonally from a maximum of 25 percent in March to a minimum of 6 percent in June. Dry gonad weights, as another measurement of gametogenic content, exhibited less monthly variation. Additionally, the semiannual cycles

TABLE 1.

Monthly mean mature gamete volume fraction ( $\bar{X}$ ) and standard deviation ( $\sigma$ ) for *P. magellanicus* from the Virginia (Va), Delaware-Maryland (De-Md), and New Jersey sampling areas.

Month	Pooled		Virginia		Delaware-Maryland		New Jersey	
	$\bar{X}$	$\sigma$	$\bar{X}$	$\sigma$	$\bar{X}$	$\sigma$	$\bar{X}$	$\sigma$
MALES								
January	55.25	14.60	50.25	14.60	53.25	13.14	65.76	5.52
February	53.95	15.70	55.47	13.89	54.83	12.35	50.42	21.47
March	66.69	14.89	71.52	8.43	62.08	18.17	—	—
April	82.10	11.25	82.54	13.93	82.61	7.62	80.27	11.62
May	58.75	35.04	84.56	14.29	26.70	31.12	71.24	16.30
June	28.01	31.96	40.55	32.28	4.12	9.95	42.71	33.18
July	38.23	29.18	40.62	33.24	37.74	27.75	35.94	26.85
August	14.65	20.21	17.82	24.47	11.95	18.33	15.15	16.66
September	41.72	27.28	43.00	29.11	40.55	26.06	—	—
October	58.85	27.01	56.68	31.94	68.29	18.14	44.00	24.63
November	42.06	32.55	31.82	29.61	48.52	33.40	—	8
December	35.48	25.86	29.73	25.62	26.82	20.25	59.27	22.46
FEMALES								
January	57.92	15.48	57.79	13.88	56.34	17.00	59.75	17.92
February	55.97	19.34	59.43	18.52	60.27	15.03	43.28	21.57
March	66.73	15.51	72.42	11.48	61.69	17.02	—	—
April	65.13	17.98	63.79	20.95	71.27	10.54	55.03	20.08
May	45.30	34.14	71.13	14.73	14.84	26.02	56.39	26.58
June	15.20	26.01	28.52	31.26	0.89	1.40	16.50	26.80
July	16.91	23.35	24.49	30.29	12.33	16.78	14.33	20.41
August	10.85	17.00	16.65	20.75	4.75	10.93	9.28	13.33
September	31.38	28.62	30.11	26.79	33.02	31.48	—	—
October	55.64	27.92	51.62	27.65	62.11	26.50	50.35	30.54
November	30.22	31.57	20.50	20.21	34.25	34.72	—	—
December	27.39	27.98	20.60	24.69	21.50	20.48	57.76	31.41

were more apparent since the increased water content present in spent gonads was removed.

Variability of gametogenesis within the mid-Atlantic Bight, as determined by the standard deviation of pooled mean mature gamete volume fractions, was quite large throughout the year (Table 1). The variability of reproductive condition did not decrease when mean mature gamete

volume fractions were calculated for individual areas and sex. Large standard deviations for the pooled volume fractions were therefore not due to pooling. In Virginia, estimated fecundity for a standardized scallop 103 mm in shell height was estimated at 39,688,000 eggs in spring and 9,938,000 eggs in fall (Table 2). In Delaware-Maryland, fecundity was estimated at 34,125,000 eggs in the spring,

TABLE 2.

Parameter estimates from regression of natural logarithm of gamete weight against constant, natural logarithm of shell height, and shell height.

Month	Parameter estimates <sup>1</sup>				Gamete wt (g)	Egg # ( $\times 1000$ )
	$\ln \beta_0$	$\beta_1$	$\beta_2$	$R^2$		
Va						
March	-28.738	7.686	-0.047	.73	7.73	
June	37.950	-9.563	0.065	.45	1.38	39,688
October	143.181	-39.158	0.383	.09	3.10	
December	39.386	-11.476	0.138	.53	1.51	9,938
De-Md						
March	-27.261	7.396	-0.050	.54	6.49	
June	6.039	-1.741	0.020	.67	1.03	33,125
October	31.373	-8.792	0.105	.16	4.21	
December	27.623	-8.340	0.112	.71	0.50	23,188

<sup>1</sup> Parameter estimates obtained by ordinary least squares for the model:

$$\ln G = \ln \beta_0 + \beta_1 \ln S + \beta_2 S.$$

and 23,188,000 eggs in the fall. The magnitude of fecundity for spring and fall spawning cycles was more consistent in the Delaware-Maryland area and was estimated to produce a greater number of eggs annually than in the Virginia area.

Scallops from shallow and deep water exhibited gametogenic development and spawning in spring and fall (Fig. 3). In spring, deep water scallops lagged one month behind shallow water scallops in the timing of gametogenic processes. In fall, the timing of gametogenic processes was very similar between water depths for both sexes. The relative fecundity of scallops, in terms of net difference in mature gamete volume fractions did not differ with depth, for either male or female scallops. A major depth related difference was that summer gametogenic activity was detected in the shallow water scallops of both sexes, but was absent from the deep water scallops (Table 3). Statistically significant differences were detected between mature gamete volume fractions of shallow and deep water samples for both sexes at the 0.05  $\alpha$  level, but not at the 0.01  $\alpha$  level (Wilcoxon paired sample test: males,  $z = -2.3368$ ,  $p < 0.0194$ ; females,  $z = -2.4581$ ,  $p < 0.0140$ ).

Estimated bottom water temperatures for the three sampling areas indicate similar annual cycles (Fig. 4). Water temperatures were generally warmest in winter months (November to January) at approximately 12–14°C and coolest in summer months (May to July) at approximately 7–8°C. Gamete development and spawning coincided with decreasing and stable temperatures in winter and spring and increasing temperatures in fall. No major deviation in mature gamete volume fractions was associated with the large decrease in temperature which occurred between January and February.

DISCUSSION

Histological examination and quantitative analysis of gonadal tissue confirmed that *P. magellanicus* from the

TABLE 3.

Samples included in the Wilcoxon paired sample test comparing gametogenic cycles of shallow and deep water scallops.

Month	Shallow		Deep	
	Date	Depth (m)	Date	Depth (m)
February	2/18	46	2/02	59
March	3/28	51	3/27	62
April	4/21	44	4/19	65
May	5/26	44	5/05	63
June	6/27	50	6/19	62
July	7/17	53	7/18	64
August	8/29	52	8/23	59
September	9/22	43	9/26	64
October	10/22	56	10/16	64
November	11/06	38	11/12	56
December	12/15	43	12/07	66

mid-Atlantic Bight underwent a semiannual gametogenic cycle as indicated by DuPaul et al. (1989). Minor temporal differences in gametogenesis existed between the three sampling areas. During spring, initiation of spawning commenced in the Delaware-Maryland area prior to spawning in New Jersey and Virginia areas. During fall, initiation of gametogenesis and spawning occurred during identical months at all sampling areas. Significant differences were detected in the gametogenic cycle of female scallops from the three sampling areas.

Shallow water scallops generally exhibited larger values of mature gamete volume fractions and additional gametogenic activity during the summer months than deep water scallops. Relative fecundity, however, was similar between depths for both male and female scallops. This result differs from previous literature which found fecundity to decrease with depth due to decreased food availability (MacDonald and Thompson 1985, 1986, Barber et al. 1988, Schiek et al. 1988). Apparently, the depth differences in this study were not large enough to demonstrate differences in relative fecundity or the relationship may not hold true for the mid-Atlantic region.

Male and female scallops within each sampling area released the majority of gametes during identical months. Such synchronous spawning is critical for reproductive success of a species which is fertilized externally (Langton et al. 1987). The statistically significant differences detected between male and female gametogenic cycles were attrib-

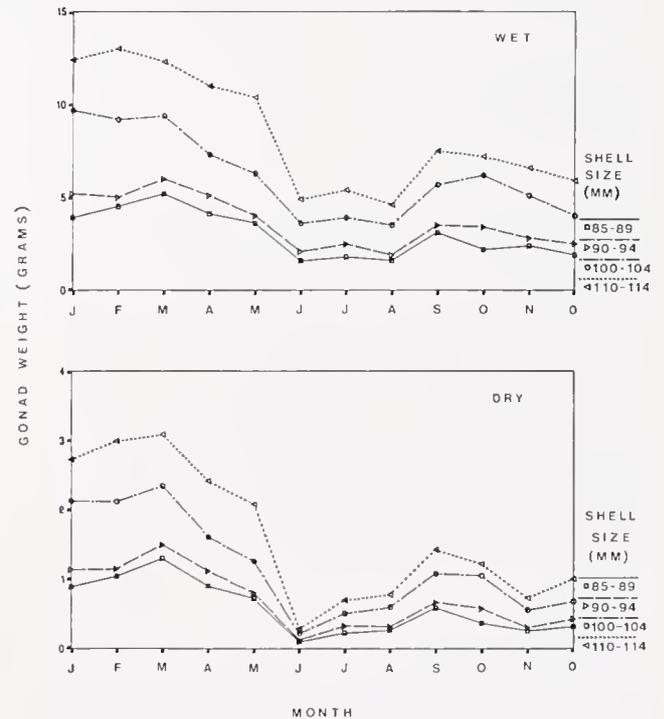


Figure 3. Pooled monthly mean wet and dry gonad weight (grams) for sea scallops of four shell height intervals (85–89 mm, 90–94 mm, 100–104 mm, and 110–114 mm).

uted to morphometric differences between sex rather than temporal differences in gametogenesis.

Spring and fall gametogenic cycles differed in magnitude and duration, with the spring cycle encompassing a greater amount of time and exhibited in greater estimated fecundity. Such inequality is typical of semiannual reproductive cycles (Comely 1974). Temperature has often been implicated as a primary environmental factor controlling the duration and timing of gametogenic events (Sastry 1966, Giese and Pearse 1974). The low uniform temperatures in spring relative to fall could therefore be responsible for the differing durations of the gametogenic cycle. Greater and more consistent fecundity in spring relative to fall may be an indication that environmental conditions in the mid-Atlantic Bight are more favorable for reproduction at that time (Newell et al. 1982, Rodhouse et al. 1984).

There was no quiescent period between the two gametogenic cycles, unlike sea scallops examined from Newfoundland (MacDonald and Thompson 1986). In addition to the two major spawnings, a small amount of gamete development and spawning in July and August as detected in Delaware-Maryland scallops. A small percentage of mature gametes were also released from New Jersey scallops in February prior to the major spawning period. The small release of spermatozoa has been referred to as dribble spawning by Newell et al. (1982) and MacDonald and Thompson (1988). The premature release of gametes may be a necessary sloughing mechanism for an animal that is reproductively mature for a prolonged period.

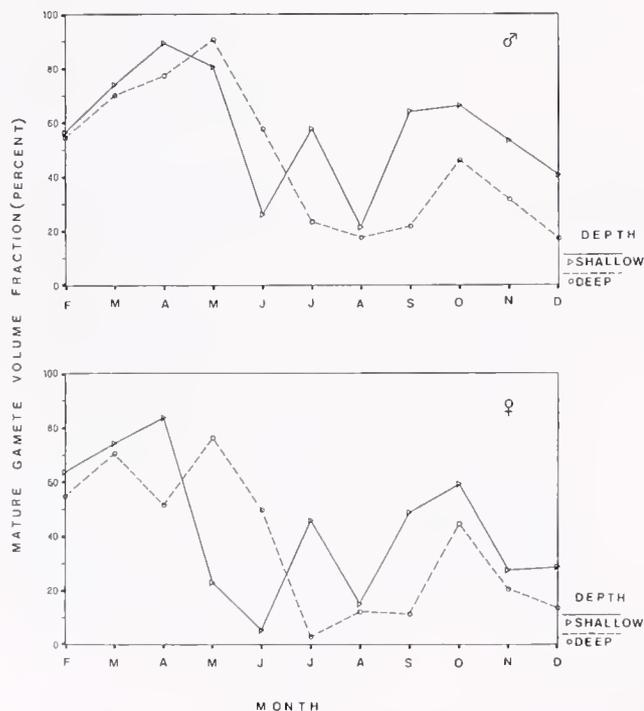


Figure 4. Monthly mean mature gamete volume fractions (percent) for male and female sea scallops from shallow and deep sampling areas.

Many of the observed differences between the gametogenic cycle of *P. magellanicus* in the mid-Atlantic and those from more northerly regions support established zoogeographic principles. As latitude decreases, there may be a shift from annual to semiannual spawning cycles, initiation of gametogenesis and spawning later in the year, less synchronous spawning, or a difference in relative fecundity (Plitzenmeyer 1965, Barber and Blake 1983, Newell et al. 1982). Sea scallops located off the coast of Newfoundland spawn annually from late August to early September (MacDonald and Thompson 1986). Sea scallops from Georges Bank also spawn annually from late September to early October (MacKenzie et al. 1978). Results from this study indicate that at the southern extent of the species range, *P. magellanicus* shifts to semiannual spawning and initiates fall gametogenesis and spawning later in the year.

In this study, the estimated fecundity of each seasonal cycle, with the exception of the Virginia fall spawn, was similar to values reported by Langton et al. (1987) and MacDonald and Thompson (1986) for sea scallops from the Gulf of Maine and Newfoundland, respectively. Since there were two spawning events per year in the mid-Atlantic Bight, however, estimated fecundity was actually much greater on an annual basis at the more southerly location. MacDonald and Thompson (1988), when comparing sea scallops from Newfoundland and New Jersey waters, also concluded that relative fecundity was greater in the southern location.

Lack of a distinct quiescent period between cycles, dribble spawning in males, and resorption of oocytes in female scallops contributed to increased variability of mature gamete volume fractions. The standard deviation of female mature gamete volume fractions in this study were larger than in the Newfoundland study (MacDonald and Thompson 1986). Thus, there appears to be less synchrony of gametogenic processes at the southern extreme of *P. magellanicus*' range. Estimated water temperatures in the mid-Atlantic Bight differ from other scallop resource areas and may be responsible for the observed deviations in the gametogenic cycle between geographic locations. High water

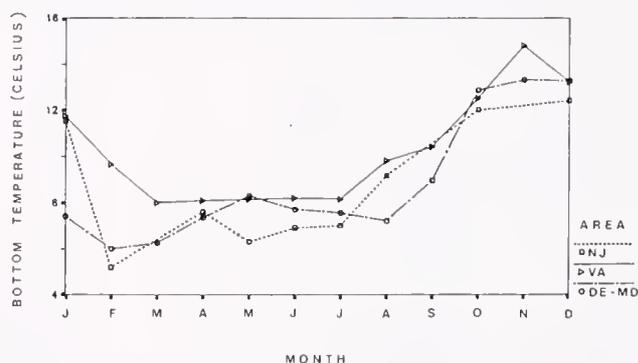


Figure 5. Monthly estimated bottom water temperature (Celsius) at the Virginia (Va), Delaware-Maryland (De-Md), and New Jersey (NJ) sampling areas.

temperatures in fall and winter in the mid-Atlantic Bight are caused by slope water intrusion (O'Reilly et al. 1987, Mountain, personal communication). At the southern region of the shelf, slope water extends onto the shelf to at least the 60 m line in fall and winter, causing an increase in bottom water temperature at that time despite the cooling and mixing of surface waters. Presence of the warm nitrate-rich slope water subsequently elevates primary production. The elevated productivity could provide the energy necessary to sustain gonadal development from December through April. Scallops on Georges Bank, without access to this energy source and subjected to cooler water temperatures, may not be energetically capable of undergoing gonadal development in the winter.

Although histological quantification of gonad tissue provides very accurate information on reproduction, it is costly and labor intensive, and thus, may be prohibitive to fishery management agencies desiring a long-term monitoring program. Morphometric changes in reproductive organs offer an accurate alternative approach. The similarity of results from morphometric measurements and histological quantification of gonadal material verified that future monitoring of gonadal development could be accurately conducted through measurement of wet or dry gonad weights.

Sea scallop management strategies that consider recruitment models or seasonal meat count adjustments related to

annual spawning event may not be consistent when a significant part of the commercially harvestable resource exhibit biannual spawning. Meat count adjustments to compensate for spawning induced changes in meat yield should follow documented seasonal reproductive patterns to be effective. However, the critical issue for management will center around whether or not both spawning events contribute to the recruitment of commercially harvestable scallops.

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## **ABSTRACTS**

### **PACIFIC COAST OYSTER GROWERS ASSOCIATION**

#### **NATIONAL SHELLFISHERIES ASSOCIATION (Pacific Coast Section)**

Forty-fourth Annual Meeting

September 27 — 29, 1990

Shilo Airport Inn

Portland, Oregon



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**MANAGEMENT AND STOCK ASSESSMENT OF THE RED SEA URCHIN (*STRONGYLOCENTROTUS FRANCISCANUS*) IN WASHINGTON STATE: PERIODIC ROTATION OF THE FISHING GROUNDS.** Alex Bradbury, Washington Dept. of Fisheries, Point Whitney Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, WA 98320.

Red sea urchins (*Strongylocentrotus franciscanus*) support a long-established commercial fishery in Washington State. During the 1988/89 season, divers landed 8.1 million pounds worth US \$2.4 million at the dock.

Red urchins exhibit several peculiar life history traits which we have attempted to address in managing this rapidly-growing fishery: they are semi-sessile and thus easily harvested at shallow depths; they distribute themselves contagiously; urchins in deep, unharvestable waters move to shallow depths near the kelp (*Neoreocystis*) line, particularly when this shallow zone has been left barren by divers; large (>120 mm) red urchins shelter conspecific recruits from predation beneath their protective "spine canopy"; overall recruitment is low (approximately 6.5%), but varies significantly between areas; growth and size frequency distribution also varies significantly between areas.

These peculiarities in urchin life history—coupled with peculiarities of diver behavior—have prompted us to discard several traditional management methods. Catch-per-unit-effort analyses, for example, can be misleading (at least over the short term) due to divers' fishing tactics and in-season urchin immigration. Life history and diver behavior considerations have led to a fishery managed via rotation of the fishing grounds on a three-year basis. Other features include a restricted season and both upper and lower size limits; these vary by area and exclude approximately 40% of the total population from harvest.

Since 1984, repeated dive surveys at 146 commercially-harvested sites have allowed us to follow the effects of the fishery and rotational management on subtidal urchin populations. Preliminary results in one area closed to fishing for 30 months indicate that repopulations to 1984 density and size-frequency levels has occurred after an intensive fishery.

**EPIZOOTIOLOGY OF HEMIC NEOPLASIA IN *MYTILUS TROSSULUS* IN WASHINGTON STATE, PART II.** Kenneth M. Brooks\* and Ralph A. Elston, Battelle Marine Research Laboratory, 439 West Sequim Bay Road, Sequim, WA 98382.

The epizootiology of hemic neoplasia (HCN) in the bay mussel is examined with field, laboratory and electrophoretic evaluations of HCN resistant populations. Challenge experiments examine the selection for resistance to this disease in an HCN enzootic area. An observed correlation between the prevalence and intensity of HCN and (mussel population density)/(water circulation) indices is examined in controlled laboratory experiments.

Field and laboratory studies have affirmed the resistance of southern California and east coast populations of the bay mussel to

HCN. Populations with *Mytilus edulis* or *Mytilus galloprovincialis* isozyme affinities suffered fewer than ten percent mortality during a one year field trial while populations of *Mytilus trossulus* experienced mortalities between 30 and 60 percent. Histology and hemocytology confirm that a significant proportion of the observed mortality is associated with HCN. Growth of all field tested populations was similar.

These studies further support the thesis that HCN is an economically important disease in Washington State and is the cause of significant mortality from settlement of plantigrades through the second year of life. Whole hemocyte challenge experiments demonstrate selection for resistance to HCN. Two and three year old mussels from an HCN enzootic area are more resistant to challenge than 0+ mussels from the enzootic area or any year class of *Mytilus trossulus* from a disease free area.

This research is supported by grants from the National Cancer Institute and the U.S. Department of Agriculture.

**GONADAL AND RELATIVE MOVEMENT DIFFERENCES WITH DEPTH OF RED SEA URCHINS *STRONGYLOCENTROTUS FRANCISCANUS* IN SOUTHEAST ALASKA.** Diane Carney, Department of Marine Science, University of California, Santa Cruz 95064.

Gonads of red sea urchins *Strongylocentrotus franciscanus* in Southeast Alaska taken during the summer from shallow subtidal sites below a surge-induced kelp fringe were found to differ from gonads of *S. franciscanus* from the same sites but at deeper depths. Gonad index was significantly higher in urchins taken from the shallows. Color and texture also differed. Urchins from the shallows tended to be brighter yellow or orange and creamy textured compared to their darker, stringier counterparts from the depths. Gonadal histological examination showed a difference in reproductive status between urchins from the two depth strata. Shallow urchins appeared to be in more active stages of gametogenesis. Deeper urchins showed a higher percentage of reproductive inactivity or degeneration.

Urchins were tagged and tracked to compare relative movements. Shallow urchins tended to be more sedentary whereas deeper urchins roamed more widely. Food availability, size, age and depth in relationship to gonadal development and relative movement are discussed.

**FEEDING JUVENILE GEODUCKS (*PANOPE ABRUPTA*) IN A SAND SUBSTRATE NURSERY: KILL A LOT, LEARN A LITTLE.** Luran R. Cole,\*<sup>1</sup> J. H. Beattie,<sup>2</sup> and D. E. Velasquez,<sup>1</sup> <sup>1</sup>University of Washington School of Fisheries, WH-10, Seattle, WA 98195; <sup>2</sup>Washington Department of Fisheries, Pt. Whitney Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, WA 98320.

Survival and growth of early post-larval geoducks in the WDF Geoduck Enhancement Project's sand substrate nurseries varies

substantially with site and season. The mobile plantigrades apparently feed from benthic diatoms, detritus and bacteria from the sand surface for several weeks after the onset of metamorphosis. At the Dabob Bay and Budd Inlet nurseries, the geoducks grow on sand with only wild algae as a food source, but food availability has only been suspected as a limiting factor in survival and growth.

Experiments during 1990 compared survival and growth in plantigrade geoducks raised on wild algae, to those grown in filtered sea water with supplemental feeding, and to nonfed controls. Supplemental food treatments included adding dried *Tetraselmis suecica* (Celsys Algal 161) in a slurry to the substrate, and suspending either *Tetraselmis* or live cultured *Chaetoceros muelleri* in the water column. Preliminary results show significant differences in growth between treatments with food available to the substrate and those with food added only to the water column.

**FEDERAL MOU'S ALLOWING IMPORTATION OF LIVE SHELLFISH: PUBLIC HEALTH, NATURAL RESOURCE, AND INTERSTATE SHELLFISH SANITATION CONFERENCE IMPLICATIONS.** F. S. Conte, Department of Animal Science, University of California, Davis, CA 95616-8521.

The Western Regional Aquaculture Consortium (WRAC) sponsored a shellfish sanitation forum in South San Francisco, California in late 1989. Participants included a national selection of shellfish sanitation scientists, federal shellfish sanitation administrators (including FDA and NMFS observers), and representatives from state health agencies and the shellfish industry from the Pacific Coast. The primary charge of the participants was to define the current status of shellfish sanitation research, define research priorities for shellfish sanitation, and address 17 Issues of significance to the health service agencies and the shellfish industry.

The WRAC Shellfish Sanitation Forum addressed the Memorandum of Understanding (MOU) established between the FDA and Chile that allows importation of live fresh shellstock from Chile into U.S. markets. From this was developed Issue #1, which addresses the MOU's with foreign governments in a broader context. Issue #1 was examined in the context of: shellfish sanitation and health safety; potential impact on domestic natural resources; and impact on the relationships between the ISSC, federal and state health service agencies, and the industry.

The agency, industry and science sections of the forum concluded that risks to public health and the natural resource do exist under the present importation protocol and that federal policy and mechanisms that established the protocol have produced a level of dissatisfaction among western industry and state agency personnel that impinges on their relationship within the Interstate Shellfish Sanitation Conference. The philosophies behind these conclusions are discussed. Also included is the series of recommendations and actions that were developed and implemented by the industry, agency, and science sections of the forum.

**GROW-OUT ASPECTS FOR THE CULTURE OF THE JAPANESE SCALLOP, *PATINOPECTEN YESSOENSIS*: IMPLICATIONS FOR THE COMMERCIAL PRODUCTION ALONG THE NORTHWEST COAST OF NORTH AMERICA.** Stephen F. Cross, Aquametrix Research Ltd., 204-2527 Beacon Ave., Sidney, B.C., V8L 1Y1.

Hatchery seed (1–2 cm & 3–4 cm) produced at the Pacific Biological Station (Nanaimo) were deployed at a grow-out site located off the northwest coast of Vancouver Island on two occasions (March, 1989 and March, 1990). Growth and survival of the Japanese scallop seed were monitored monthly through to market size. Design of this program permitted assessment of growth characteristics influenced through biotic factors (stocking densities, plankton availability, fouling, infection), by abiotic factors (physical and chemical oceanographic conditions), and by culture method (pearl/lantern nets; ear-hanging).

Stratified sampling at depths of 5, 10, 15, 20, and 25 metres indicated a decrease in both survival and growth rates below 15 metres. Significant difference in growth rates with depth were not observed, however, until the animals exceeded 45–50 mm. Phytoplankton community structure (cell size/abundance) within the water column was related to the differential growth pattern.

Size of seed and timing of deployment also reflected significant differences in growth and survival. Seasonal availability of plankton and ingestion capability were attributed to the observed differences.

Water column stability, including high salinity (over 30 ppt), low temperatures (6–10°C), slow currents, and minimal wave action affecting the overlying longline system were considered important factors contributing to the minimal effects noted in the density-dependent aspects of this study. With lantern net densities as high as 50 animals/layer significant mortality was not observed until animals exceeded 65–70 mm. Growth rates were also slightly depressed once this size was attained.

Proper site selection, in conjunction with the demonstrated hardiness of the Japanese scallop (overall grow-out mortality to market size of less than 10%; growth of 6–10 mm/month), makes culture opportunities for this species very attractive for the Pacific northwest.

**MANAGEMENT OF CALIFORNIA'S SEA URCHIN FISHERY.** Christopher M. Dewees, Department of Wildlife and Fisheries Biology, Sea Grant Extension Program, University of California, Davis, CA 95616-8751.

The fishery for red sea urchins, *Strongylocentrotus franciscanus*, began in 1972 in southern California and landings ranged from 10 to 24 million pounds from 1976 through 1984. Fishery management was minimal. During 1985 the fishery expanded to northern California. Landings and the number of divers quickly doubled. Concern about the resource led to legislation establishing a \$0.005 per pound landing tax to fund management, research, and enhancement projects. Cooperative fishery management deci-

sion making by the industry and California Department of Fish and Game led to a series of increasingly restrictive management measures including limited entry, minimum size limits, and time closures. Statewide landings continued to increase in 1989 to 51 million pounds despite drops in catch per unit effort in northern California and increased regulation. More restrictive regulations began in June 1990. It is unclear how effective these measures will be. Increased research on urchin population dynamics and implementation of innovative cooperative management schemes will be needed to sustain a healthy fishery.

#### **SHELLFISH AND MARINE PLANT AQUACULTURE IN BRITISH COLUMBIA. CONFLICTS AND SOLUTIONS.**

**Frances V. Dickson,\*** Department of Fisheries and Oceans, Vancouver, B.C. V6B 5G3; **R. K. Cox,** R.P. Bio., Ministry of Agriculture and Fisheries, Victoria, B.C. V8V 2Z7; **N. Bourne,** Department of Fisheries and Oceans, Nanaimo, B.C. V9R 5K6.

The shellfish aquaculture industry in British Columbia is dominated by production of Pacific oysters (*Crassostrea gigas*). There is growing participation in intertidal manila clam (*Tapes phillipinarum*) culture due to diminishing supplies of wild clams and in development of Japanese scallop (*Patinopecten yessoensis*) off-bottom culture. Marine plant culture continues to develop slowly with recent interest shown in culturing Nori (*Porphyra spp.*).

Development of these culture operations has been hampered by siting conflicts particularly for clam culturists due to impacts on commercial and recreational fishermen who wish to harvest wild clams on the same beaches and for scallop and nori farmers due to conflicts over anchorages, fishing grounds or navigational routes.

Joint government programs have been developed to provide special reserves for recreational clam fishermen. A co-operative federal/provincial government program is being finalized allowing for structured development of clam culture to complement the wild clam fishery.

Coastal Resources Information Studies initiated by the government of British Columbia have been completed for five zones in the province. Following consultation with user groups and government agencies, the maps produced identify conflict areas such as critical fish habitats, underwater archaeological sites, parks, Indian Reserves, anchorages, navigation channels and important fishing grounds where initiation of aquaculture activities will be limited or prohibited.

#### **CONTROLLING BURROWING SHRIMP IN WASHINGTON COASTAL ESTUARIES: A NEW APPROACH.**

**Brett R. Dumbauld,\*** **David A. Armstrong,** and **Robert F. Donnelly,** School of Fisheries, University of Washington, Seattle, WA 98195.

An investigation into the ecology of the mud shrimp *Upogebia pugettensis* and ghost shrimp *Callinassa californiensis* in Willapa Bay, Washington was initiated in 1988. Previous and on-

going studies have focused on the effects of the insecticide carbaryl, which is applied to oyster culture grounds to kill these burrowing shrimp. Alternatively, we chose to investigate the life history and ecology of the shrimp and its interaction with oyster culture practices and control procedures, hoping to find an alternative control measure or at least optimize the existing pesticide program to best manage the problem with the fewest environmental impacts.

While the current practice of spraying carbaryl to kill adult and juvenile shrimp on affected oyster beds may solve the problem in a given oyster growing cycle (2 to 3 years), we have found newly settled juveniles of at least one species of shrimp, *Callinassa californiensis*, back in the sprayed area within 2 weeks of spray. Thus, factors affecting recruitment and seasonal timing of settlement are critical, and ultimately the ability to apply the control measure more than once during the oyster growing cycle would be desirable. We collected temporal life history information on both species of shrimp and carried out recruitment experiments in 1989 and 1990. In addition, we have initiated a study on the possibility of using electrofishing gear to control the shrimp, a technique that has the potential of eliminating some of the seasonal and environmental time constraints currently imposed by pesticide restrictions.

#### **GROWTH AND STATUS OF ABALONE AQUACULTURE IN CALIFORNIA.** **Earl E. Ebert,** California Department of Fish and Game, 2201 Garden Rd., Monterey, CA 93940.

The impetus to cultivate abalone, *Haliotis*, spp., endemic to California began in 1964 at Morro Bay, located in Central California. Four years later the first commercial abalone hatchery in California, designed specifically for that purpose, became operational. The ensuing years witnessed a high level of interest in cultivation of abalone, prompted largely by a declining commercial fishery, high market demand and value. Presently two hatcheries are operating at full capacity, with a profit margin, and undergoing further expansion. At least four other hatcheries are in various production stages, while a number of companies are in the research and development phase. Three species are under cultivation and being marketed: the red, *Haliotis rufescens*, green *H. fulgens* and pink, *H. corrugata* abalone. *H. rufescens* constitutes the bulk of production, exceeding 95%. Much of the progress of the expanding abalone aquaculture industry in California is attributable to determined private entrepreneurship, comprised of individuals possessing a true pioneering spirit. University researchers, supported by California Sea Grant, have made important contributions to the science of abalone cultivation. Also, the California Department of Fish and Game's Marine Resources Laboratory contributed to the overall development of technological as well as biological procedures for abalone cultivation. Hatchery produced abalone are grown-out in either land-based tanks or in-the-sea barrels that are suspended from a raft or wharf. Animals in the barrel culture system are harvested at about 6–7 cm shell lengths.

Those produced in the land-based tanks are harvested at about 8 cm shell lengths. These abalone are approximately 2½ years old.

**POPULATION STUDIES OF RED AND PURPLE SEA URCHINS (*STRONGYLOCENTROTUS FRANCISCANUS* AND *S. PURPURATUS*) ALONG THE CALIFORNIA COAST.** Thomas A. Ebert,\* S. C. Schroeter, and J. D. Dixon, Department of Biology, San Diego State University, San Diego, CA 92182-0057.

A standing stock is a function of settlement, growth and survival. We are attempting to measure these important life history attributes in red and purple sea urchins along the California coast to provide information that is needed for resource management.

Settlement of sea urchins on scrub brush collectors has been monitored at selected sites along the California coast starting in late February 1990. A major settlement event was observed in April 1990 and since this event, settlement has been from very low to zero. More settlement occurred in the San Diego area than elsewhere in California.

Growth of intertidal populations of both red and purple sea urchins was determined using tetracycline tagging, which provides good estimates for animals that have test diameters greater than about one centimeter. Red sea urchins at San Nicolas Island require about 10 years to grow to 8 cm. Purple sea urchins at Arena Cove grow to about 5.1 cm in 10 years. Growth combined with size data can be used to estimate natural mortality rates, which are about 10 to 15% per year.

**COCCIDIOSIS OF CALIFORNIA ABALONE, *HALIOTIS* SPP.** Carolyn S. Friedman, California Department of Fish and Game, Fish Disease Laboratory, 2111 Nimbus Rd., Rancho Cordova, CA 95670.

During an investigation of mass mortality of black abalone, *Haliotis cracherodii*, in central and southern California, coccidian parasites were observed in nephridia of moribund and clinically normal animals. Microscopic examination of 5 spp. of abalone from California (red, pink, green, black and flat) and 1 spp. of abalone from Washington state and British Columbia, Canada (pinto) revealed that the coccidians occur in all spp. (>1.9 cm maximum length) throughout California. However, the parasites were not observed in pinto abalone from Washington state or B.C., Canada. Coccidian parasites were observed in both the left and/or right kidneys and did not elicit an inflammatory response in most abalone examined in our survey.

Regression analysis revealed no correlation ( $r < 0.27$ ) between intensity of coccidian infection and condition of the host ((total wt.—shell wt.)/total wt.) of both apparently normal and moribund (shrunken and weak) abalone from California. Thus, the parasites do not appear to adversely affect the abalone from California. Elevated temperature appears to accelerate black abalone mortality more than starvation as evidenced by laboratory studies.

Controlled laboratory challenges of pinto abalone with coccidian parasites by injection and cohabitation are currently in progress.

**PRODUCTION OF TETRAPLOID EMBRYOS IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*: COMPARISON AMONG DIFFERENT APPROACHES.** Ximing Guo,\* Ken Cooper,<sup>1</sup> William K. Hershberger, and Kenneth Chew, School of Fisheries WH-10, University of Washington, Seattle, WA 98195. <sup>1</sup>Coast Oyster Company, Quilcene, WA 98102.

Tetraploid induction in the Pacific oyster, *Crassostrea gigas*, is being studied in our laboratory. Methods to produce tetraploids tested so far include cell fusion, polar body I blocking, mitosis I blocking, and gynogenetic activation.

Zygote-zygote fusion was achieved, but its feasibility in tetraploid induction is greatly limited by technical complexity and low fusion efficiency. Polar body I blocking produced variable levels of triploids, tetraploids and aneuploids. Cytogenetic analysis revealed that polar body I blocking resulted in an abnormal tripolar segregation of chromosomes at meiosis II. Moderate levels of tetraploid embryos (8–44%) were obtained with blastomere fusion at the 2-cell stage and heat shock induced blocking of mitosis I. Very high levels (80–100%) of tetraploid embryos were produced by blocking the release of both polar body I and polar body II in eggs fertilized with sperm which were genetically inactivated with ultraviolet irradiation. Preliminary data indicated that all induced tetraploids had very limited viability.

**DEVELOPMENTAL ACTIVATION OF PACIFIC OYSTER, *CRASSOSTREA GIGAS*, EGGS.** Ximing Guo,\* William K. Hershberger, Ken Cooper,<sup>1</sup> and Kenneth Chew, School of Fisheries WH-10, University of Washington, Seattle, WA 98195. <sup>1</sup>Coast Oyster Company, Quilcene, WA 98102.

Developmental activation of Pacific oyster, *Crassostrea gigas*, eggs was studied using parthenogenetic and gynogenetic techniques. Parthenogenetic activation was tested with ammonium hydroxide, calcium chloride, and hypotonic seawater. All treatments except calcium chloride induced some activation of eggs. High proportions (up to 84%) of egg activation were more often obtained using combinations of ammonium hydroxide and hypotonic seawater. Development of activated eggs usually stopped after the release of the two polar bodies. The reproducibility of all parthenogenetic techniques was low, and egg quality might be a major factor.

Gynogenetic activation was tested with toluidine blue and ultraviolet (UV) treated sperm. Complete inactivation of the sperm chromosomes was observed in both toluidine blue and UV treatment, but results from toluidine blue treatment were less consistent than that from UV treatment. UV irradiation of sperm produced a clear 'Hertwig effect' as measured by survival of larvae to D-stage. Eggs fertilized with inactivated sperm developed uniformly as haploids. Among the approaches tested in this study,

UV irradiation of sperm appears to be the most effective method for developmental activation of Pacific oyster eggs.

**DOES VARIANCE IN REPRODUCTIVE SUCCESS LIMIT EFFECTIVE POPULATION SIZES OF MARINE ORGANISMS? A PROPOSED TEST IN THE DABOB BAY POPULATION OF PACIFIC OYSTERS, USING ENZY-MATIC AMPLIFICATION OF MITOCHONDRIAL DNA.**

**Dennis Hedgecock,\* Keith Nelson, and Michael A. Banks,** Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923.

Estimates of long-term effective population sizes ( $N_e$ ) derived from studies of biochemical genetic variation are several orders of magnitude less than actual population sizes for most organisms, including marine fish and shellfish species. Two independent estimates of  $N_e$  for the American oyster *Crassostrea virginica*, for example, are on the order of  $10^5$ , in contrast to annual landings of  $10^{10}$  individuals. Large variance in reproductive success (number of offspring per parent contributed to subsequent breeding populations), made possible by great fecundity and mediated by sweepstakes recruitment success of broods, would explain such discrepancies. This hypothesis predicts (1) lower genetic diversity in cohorts of larvae or spat than exists in the adult breeding population and (2) temporal variance in allelic frequencies owing to random genetic drift.

Drift of allozyme frequencies and a  $10^4$ -fold discrepancy between effective and actual population sizes have been observed for Pacific oysters *C. gigas* in Dabob Bay, WA. We have now used the polymerase chain reaction (PCR) to amplify maternally inherited mitochondrial DNA sequences from single larvae, spat and adult Pacific oysters. We are currently sequencing these genes to determine polymorphic nucleotide sites suitable for typing using sequence-specific oligonucleotide probes. With these PCR-based techniques we propose to obtain the information on genetic diversity of larval and adult Pacific oysters in Dabob Bay that is critical to testing the hypothesis that variance in reproductive success is large in this natural bivalve population.

**BODY SIZE AT HARVEST AND SEX RATIO OF PEDIGREED PACIFIC OYSTERS *CRASSOSTREA GIGAS* FROM CONTROLLED CROSSES.** **Dennis Hedgecock,\*** Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923; **Ken Cooper,** Coast Oyster Co., Quilcene, WA 98376; **Bill Hershberger,** and **Ximing Guo,** School of Fisheries, University of Washington, Seattle, WA 98195.

A collaborative project of the USDA's Western Regional Aquaculture Consortium (WRAC) has made a series of 8 experimental crosses of Pacific oysters, two  $8\sigma \times 3\text{f}$  factorial and six  $8\sigma \times 3\text{f}$  hierarchical, yielding a total of nearly 192 families. Progeny have been set on clutch, randomized on long lines and

deployed to growout areas in Puget Sound, WA, and Humboldt Bay, CA. Data on wet and dry body weights and sex ratios at 16 mos. age for 22 families from the first cross reveal substantial variation in all traits. Full-sibs grown on different cultch can vary significantly in body size; after pooling family data across cultch, the sire, dam and interaction components of variance in body size are all highly significant. Fitting of log-linear models to data on family sex ratios indicates that sex ratio depends on sire. Similar data for other crosses being harvested in June 1990 will be presented.

These data suggest that there is substantial additive genetic variation for body size at harvest, thus a high heritability for growth and a reasonable expectation for response to selection for increased growth rate. Estimates of genetic components of variance and heritability from the present data are, nevertheless, fairly imprecise owing to the limited number of families in each experimental cross. The data on sex ratio support previous published studies suggesting that sex is determined by a relatively small number of autosomally inherited genes having male- and female-determining alleles.

**GROWTH OF JUVENILE ABALONE, MUSSELS, AND SPOT PRAWNS IN AN EXPERIMENTAL POLY-CULTURE.** **John W. Hunt,** Institute of Marine Sciences, University of California, Santa Cruz, CA 95064.

Juvenile red abalone (*Haliotis rufescens*), mussels (*Mytilus californianus*), and spot prawns (*Pandalus platyceros*) were cultured in monocultures and in polyculture. All groups were fed three diet rations: unfiltered seawater only; unfiltered seawater plus additions of benthic diatoms, phytoplankton, and shrimp meat; and unfiltered seawater plus a larger ration of the three foods.

Growth of abalone and prawns was significantly greater in monoculture treatments (0.48 g and 0.72 g mean weight increase, respectively) than in polyculture (0.28 g and 0.41 g mean weight increase, respectively). Growth increased significantly with amount of food supplied (0.15 g vs 0.60 g mean abalone weight increase for lowest and highest rations, respectively; and 0.21 g vs 0.88 g mean prawn weight increase for lowest and highest rations, respectively). Mussels grew slowly (0.04 g mean weight increase) in all treatments due to insufficient supply of algal food, and their growth was not significantly affected by any experimental manipulations. Food quantity and culture treatment produced a significant interactive effect on shellfish growth (ANOVA  $p < 0.001$ ). Despite presumed differences in feeding strategies among the three species, overlap in the utilization of food was suggested as the reason for decreased growth of abalone and prawns in polyculture relative to monocultures. Abalone were found to be capable of ingesting and assimilating shrimp meat that was intended as food for prawns in the polyculture system.

**OVERWINTERING GROWTH OF JUVENILE JAPANESE SCALLOPS *PATINOPECTEN YESSOENSIS* IN PEARL NETS; EFFECTS OF DENSITY AND WAVE EXPOSURE.**

**Brian C. Kingzett\*** and **N. Bourne**, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

Japanese scallop (*Patinopecten yessoensis*) seed greater than 25 mm were placed into 30 cm<sup>2</sup> pearl nets for secondary nursery culture during October. Nets were loaded at 25, 50, 100, and 150 scallops per net and placed out in Departure Bay, Nanaimo, B.C. Pearl nets were suspended at a depth of four meters in two separate locations, one stable and one subject to wave exposure. Salinity, temperature and secchi depth were measured and recorded daily. Scallops were sampled every two months for a six month period until such time as seed are normally moved into final grow-out culture in lantern nets or earhanging. Shell height, mortality and shell deformity were measured.

Growth was inversely related to density. Growth rates in replicates exposed to wave action were depressed. High survival was experienced in all replicates (>95%) regardless of density until the final sampling period (March–April). At this time maximum mortality and deformity appeared only in the nets exposed to wave action and with the highest density of scallops (100–150).

**COMPARISON OF SURVEY METHODS FOR SAMPLING POPULATIONS OF NORTHERN ABALONE, *HALIOTIS KAMTSCHATKANA*.** **Gretchen O. Kruse**, Huxley College, Western Washington University, Bellingham, WA 98225.

Numerous abalone population surveys used to date have been unable to determine the degree of population fluctuations over time. For this reason a population of Northern abalone, *Haliotis kamtschatkana* (Jonas), was sampled using three different survey methods over a period of three months during the spring of 1990. The three different survey methods were tested in order to determine variability and accuracy of each method. Methods tested were random daytime search, daytime grid search and random night-time search. Quadrats sampled were in known abalone habitat on level seabed consisting of large boulders interspersed with gravel. Counts and shell length measurements of all abalone found within the quadrats were recorded.

The three survey methods yielded significant differences in density of abalone during different months. Density ranged from 0.54 abalone/m<sup>2</sup> to 0.63 abalone/m<sup>2</sup>. Utilization of daytime random search when surveying this type of abalone habitat not only seems to provide representative data on population densities but is also the most convenient and least expensive survey method.

**DEVELOPMENT OF THE COMMERCIAL AQUACULTURE OF THE SUMINOE OYSTER (*CRASSOSTREA RIVULARIS*).** **Chris J. Langdon\*** and **Anya M. Robinson**, Hatfield Marine Science Center, Oregon State University, Newport, OR 97265.

The Suminoe oyster has been introduced from Japan in several localities on the West coast, USA. The species is not widely cultivated, despite its reported high growth rate in the Yaquina Bay, Oregon. We have examined the potential value of the Suminoe oyster as a commercial species on the West coast, USA.

In the Yaquina Bay, female Suminoe oysters became sexually mature in late summer with peak gonadal maturation occurring in October. In contrast, female Pacific oysters were mature in May with peak gonadal maturation occurring in September. Sexual maturation of adult Suminoe oysters could be successfully accelerated by collecting broodstock oysters from the Yaquina Bay in mid-March and holding them in flowing seawater at 20°C for 4 to 6 weeks. Conditioned broodstock oysters were spawned by subjecting them to 1–2 hours aerial exposure followed by submergence and a water temperature increase from 20°C to 30°C.

Larvae were successfully raised to setting and metamorphosis by culturing them for three weeks in 0.7 µm-filtered seawater (20 ppt and 25°C) on a mixed diet of the flagellate *Pseudoisochrysis paradoxa* and the diatom *Chaetoceros calcitrans*. Growth of planted hatchery-raised Suminoe spat was superior to that of same-age Pacific oyster spat in several localities on the West coast.

Comparative tests indicated that the appearance of the Suminoe oyster on the half-shell was preferred to that of the Pacific oyster; furthermore, the taste and texture of Suminoe oysters stewed in milk or broiled were rated equal or superior to that of Pacific oysters.

**POTENTIAL AND LIMITATIONS OF ABALONE (*HALIOTIS* SPP.) CULTURE IN THE PACIFIC NORTHWEST.**

**Amy Leitman**, Washington Department of Fisheries, Pt. Whitney Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, WA 98320.

Major advances have been made in the last two decades in abalone (*Haliotis* spp.) cultivation. Several California facilities have succeeded in making red abalone (*H. rufescens*) aquaculture a profitable industry, however, they cannot sufficiently supply the avid market. With limited amount of suitable locations in California for additional facilities, alternative sites in other states or countries are imperative for expansion. Washington and British Columbia, Canada offer suitable locations for abalone hatcheries and grow-out facilities for both the red abalone and the more northerly pinto abalone, (*H. kamtschatkana*). A culture product from the Pacific Northwest, could help supply the high demand world market.

Pressing questions are addressed about the introduction of the red abalone into Washington versus the utilization of the indigenous pinto abalone for aquaculture, the feasibility of culturing the red abalone in colder temperatures, the availability of a continuous food supply, and the political obstacles that are presently deterring culture.

**DUNGENESS CRAB, *CANCER MAGISTER*, RECRUITMENT VARIABILITY AND EKMAN TRANSPORT DURING THE PELAGIC LARVAL PHASE.** Robert A. McConnaughey\* and David A. Armstrong, School of Fisheries, University of Washington, Seattle, WA 98195; B. H. Hickey, School of Oceanography, University of Washington, Seattle, WA 98195.

Commercial stocks of the Dungeness crab, *Cancer magister*, fluctuate widely along the Pacific Coast of the United States. Previous attempts to explain this interannual variability have relied on time-lagged relationships between physical features of the environment during the planktonic larval phase and subsequent commercial landings. These efforts have been largely unsuccessful, in part, because the multi-year class composition of the catch is not accounted for by a simple time lag (typically 3 or 4 years).

We examine the influence of environmental variability on spatial patterns and year class strength measured at the time of settlement to the benthos using six years of trawl survey data collected along the coast of Washington state. August estimates of early instar abundance within two coastal estuaries and the adjacent nearshore are compared with cross-shelf transport during the preceding 3- to 4-month pelagic larval phase. Inferences are based on time series of corrected Ekman transport vectors obtained from calculated pressure fields for the survey area. Results indicate that high levels of Dungeness crab settlement are associated with persistent landward transport during the final months of the larval period. This condition will tend to minimize cross-shelf advective loss of larvae while promoting retention near suitable juvenile and adult habitat. Relatively low levels of recruitment followed sustained northward transport during the pelagic larval phase. Intermediate levels of settlement occurred when sustained northward transport was moderated by a persistent landward drift. We find no evidence for sustained westward Ekman transport to account for progressive offshore transport through ontogeny and suggest a reevaluation of the prevailing, albeit problematic, model for Dungeness crab recruitment is in order.

**AN OVERVIEW OF CRAB MITIGATION IN GRAYS HARBOR, WASHINGTON.** Kay A. McGraw,\* Fred C. Weinmann,<sup>2</sup> and David A. Armstrong<sup>1</sup>. <sup>1</sup>School of Fisheries, University of Washington, Seattle, WA. <sup>2</sup>Environmental Protection Agency, Region 10, Seattle, WA.

Mitigation for dredging impacts to Dungeness crabs in Grays Harbor, WA began in April 1990 after several years of planning, negotiation, and discussion among the U.S. Army Corps of Engineers, state and federal agencies, scientists, and crab fishermen. The process leading to mitigation involved basic research on crab biology and life history, crab entrainment studies, dredge modification, site evaluation, implementation and interpretation of environmental regulations and policies, and many other components.

A perspective of the process is presented, along with some of

the various viewpoints, obstacles, and accomplishments encountered that led to the present solution. Some economic considerations are also provided.

**SPORE ULTRASTRUCTURE OF A HAPLOSPORIDAN PARASITE OF RAZOR CLAMS, *SILIQUA PATULA*.** James D. Moore\* and Ralph A. Elston, Battelle Marine Sciences Laboratory, 439 West Sequim Bay Road, Sequim, WA 98382.

A moribund razor clam collected in 1986 from Mocrocks Beach, Jefferson County, Washington was found to harbor a massive systemic infection of an unidentified haplosporidan parasite. Recent concern regarding the occurrence and spread of haplosporidan parasites associated with commercial Pacific bivalves prompted ultrastructural descriptions of, and taxonomic studies on preserved spores from this animal.

Transmission electron microscopy demonstrated spore characteristics unique to the family Haplosporidiidae, including the presence of an operculum and haplosporosomes. For scanning electron microscopy (SEM), intact spores were isolated by both trypsinization and mechanical disruption of clam neck tissue. Average spore size by SEM was  $5.8 \times 2.6 \mu\text{m}$ . Two long filaments, one extending from each end of the spore, appear to be extensions of epispore cytoplasm rather than derivations of the spore wall, which is devoid of protruding ornamentation. These characteristics allow tentative assignment in the genus *Minchinia*. The spore dimensions and novel host suggest that the parasite represents a new species of the genus. A unique structural configuration was observed in some sporocysts in which epispore cytoplasm filaments appeared to wrap around the perimeter of the aggregation of individual spores.

Approximately 20 additional razor clams with undescribed protozoan infections may represent earlier ontogenetic stages of the same haplosporidan parasite.

This work was supported by the Washington State Department of Fisheries.

**STATUS AND TRENDS OF SCALLOP AQUACULTURE ON THE WEST COAST (NORTH AMERICA).** Yun-Wook Rhee, School of Fisheries, University of Washington, Seattle, WA 98195.

At least four species of scallops: Weathervane (*Pecten caurinus*), rock (*Crassadoma gigantea*), spiny (*Chlamys hastata*), and pink (*Chlamys rubida*) exist for potential aquaculture on the West coast of North America. Research on natural spat collection culture has been active in the 70's and 80's in California, Washington, and Alaska. In Canada, research on hatchery based culture has been completed on the four species as well as the imported Japanese scallop (*Patinopecten yessoensis*) in the 80's. However, in spite of the extensive research in the past, there are no active

commercial scallop farmers on the West coast. The reasons for slow technology transfer, current status, and future prospect of scallop farming on the West coast is presented.

**SURVIVAL AND GROWTH OF GEODUCK, (*PANOPE ABRUPTA*) JUVENILES IN A SAND SUBSTRATE NURSERY, BUDD INLET, WASHINGTON.** Dean E. Richardson\*<sup>1</sup> and J. H. Beattie,<sup>2</sup> <sup>1</sup>The Evergreen State College, Olympia, WA 98502; <sup>2</sup>Washington State Department of Fisheries, Point Whitney Laboratory, Brinnon, WA 98320.

We transported plantigrade geoducks from the Point Whitney Shellfish Hatchery to a pilot nursery site at the Department of Natural Resources pier, Budd Inlet, WA. We planted approximately 4 million plantigrades in each of 4, 10 foot (3 meter) diameter sand based nursery modules. We took quantitative replicate random samples, and used microscopic examination in order to estimate survival and growth. Growth rates averaged 0.39 mm per week over the first two months in the nursery; survival during this same period was 31.6%.

The survival data from this pilot nursery indicate superior results compared with the nursery at Point Whitney; growth rates remained comparable between the two sites. Budd Inlet would make an excellent location for an expansion nursery.

**RESEARCH AND DEVELOPMENT INITIATIVES SUPPORTING A MANILA CLAM CULTURE INDUSTRY IN BRITISH COLUMBIA.** W. G. Roland\* and P. Gubbels, Ministry of Agriculture and Fisheries, Aquaculture and Commercial Fisheries Branch, Parliament Buildings, Victoria, B.C. V8W 2Z7.

Recent industry interest in the culture of Japanese littlenecks has resulted in a need for information on site assessment, production, and economic aspects of a clam farming enterprise. Recent ministry initiatives are: 1. pilot-scale investigations of growout on a variety of beach sites, comparing several husbandry methods. Siting information is gained from an initial survey of the substrate, predators and existing stocks. Production data are compared as effected by tide heights, seeding densities, and predator exclusion, and 2. an estimated costs and returns study for a clam growout enterprise. Variables considered are the farm resource base, production and harvesting practices. A best estimate scenario is used as a basis for comparing the effects on farm income of varying clam prices, seed survival and harvesting costs.

**PROCEDURES FOR MASS HARVEST AND QUANTIFICATION OF JUVENILE *HALIOTIS RUFESCENS*.** Dehra J. Sommerville,\* David L. Eagle, and Joan M. Ford, The Abalone Farm, Inc., Cayucos, CA 93430.

The Abalone Farm, Inc. (AFI) is a commercial abalone grower

located in Central California. Recent increases in AFI's production capacity have resulted in the need to replace production procedures relying on hand harvesting and counting, with more efficient methods for mass harvest and counting of juvenile abalone. The production system employed at AFI includes a hatchery operation and a grow-out system. In the hatchery, post larvae are reared to 8 mm and selectively harvested by size for grow-out. The mass harvest method developed by AFI consists of four phases: inventory harvest, size sorting, quantification of harvest, and redistribution by size class. To allow mass harvest abalone in the production tanks are anesthetized *in situ* to minimize handling stress. The abalone are harvested using salt water spray and collected for sorting. The harvest is sorted into discrete size classes using 2 mm graduated sizing screens. The quantification of the harvest is achieved by weight extrapolation from a 1% subsample. To achieve the required accuracy, the critical step in the quantification process is the removal of excess water without damaging the inventory. The inventory size classes are either redistributed to hatchery tanks or transferred to grow-out tanks. The implementation of this method on a large scale has retained a count accuracy of  $\pm 2\%$  with an increase in harvest productivity, from an average of 800 abalone per man-hour in 1988, to 3,000 per-man hour in 1989.

**DEVELOPMENT OF JAPANESE LITTLENECK (*TAPES PHILIPPINARUM*) HABITAT BY BEACH GRAVELING.** Doug Thompson\* and W. Cooke, Washington Department of Fisheries, Point Whitney Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, WA 98320.

The Washington Department of Fisheries (WDF) is using a process known as beach graveling to create new clam habitat on mud and combination mud/sand beaches. Previously graveled plots, established between 1973 and 1983 have demonstrated relatively low levels of clam production (9.0 clams/m<sup>2</sup>/yr). Limiting factors are silt deposition and erosion of gravel plots, predation by crab, fish and moon snail, and thick layers of gravel (>15 cm) producing hydrogen sulfide gas. Methods used to reduce the impact of these factors on clam production are discussed.

In 1989, six 0.3 acre gravel plots were established at Oakland Bay, near Shelton, WA. Treatments of 100% gravel and a 50/50 ratio of gravel to crushed oyster shell at layer depths of 2.5 to 10.0 cm are being tested to determine the best combination for clam settlement, growth and survival. In 1990 similar treatments will be tested on nine 500 m<sup>2</sup> experimental plots. Temporal changes in species composition and abundance will be determined for all bivalve species. The impact of graveling will also be determined for other marine invertebrates that are important prey for juvenile marine fish communities.

**THE EFFECTS OF ADDING CRUSHED OYSTER SHELL TO A GRAVEL BEACH ON NATURAL RECRUITMENT OF MANILA CLAMS, *VENERUPIS JAPONICA*.** Derrick R. Toba\* and K. K. Chew, University of Washington, School of Fisheries WH-10, Seattle, WA, 98195; D. Thompson, Washington Department of Fisheries, Point Whitney Shellfish Lab, 1000 Point Whitney Road, Brinnon, WA 98320.

A site at Bywater Bay, Hood Canal was constructed to test whether the addition of crushed oyster shell to a previously gravelled plot would increase the production of Japanese littlenecks (*Venerupis japonica*). The purpose of adding crushed oyster shell is to neutralize the sediment pH, counter the effects of gravel compaction and increase the surface area for settlement of clam larvae. Macroalgae was removed from the surface of eighteen 2 × 4 meter plots and three replicates of the following treatment were established: 1) control, no substrate alteration beyond algae removal; 2) no substrate alteration beyond algae removal with 5,000 seed clams added; 3) substrate rototilled to a 10.0 cm depth; 4) substrate rototilled to a 10.0 cm depth with 5,000 seed clams added; 5) a 5.0 cm layer of crushed oyster shell rototilled into the substrate to a 10.0 cm depth; 6) a 5.0 cm layer of crushed oyster shell rototilled into the substrate to a 10.0 cm depth with 5,000 seed clams added. Rototilling a 5.0 cm layer of shell to 10.0 cm results in a 50/50 ratio of shell to existing gravel. The seed clams planted in this study ranged from 6–8 mm in size and planted at a density of 600–800 juveniles/m<sup>2</sup>. One half of each plot was covered with 7 × 14 mm predator exclusion netting. At three month intervals, five core samples (800 cm<sup>3</sup>) will be randomly sampled on each half of the plot to measure the rate of natural recruitment, and growth and survival of clam seed. This paper will present preliminary results of rates of natural recruitment to each of the treatments.

**IMPROVING OYSTER SPAT SURVIVAL.** Paul S. Williams\* and K. K. Chew, Department of Aquaculture, School of Fisheries, University of Washington, Seattle, WA 98195; R. N. Steele, Rock Point Oyster Company, Quilcene, WA 98376.

In a series of ongoing experiments initiated in May 1990, techniques used to increase survival of pacific oyster *Crassostrea gigas* spat set in remote setting operations were evaluated. The techniques and factors affecting spat survival chosen for the study include: season, length of time in the setting tanks, tidal height, sprinkling with water, shading, and submersion from floats. Spat were set in commercial remote setting tanks at 25°C and fed cultured algae throughout the setting period. Survival of spat was monitored from 3 days after setting to 60 days. Preliminary results suggest that survival of spat was greater for those kept in setting tanks for 6 days before transfer to long lines compared to those kept in the tanks for 3 days; however, a setting period of 14 days yielded no further increase in survival and may even be detri-

mental. Further tests to be reported here will determine if the increase in survival for those held for 6 days can be expected year round or if the duration of setting time should vary seasonally. In addition, the results of studies investigating postsetting survival will be presented.

**CRABBY PREDATORS: SCOURGE OF THE INTERTIDAL BEHRENS.** Sylvia Yamada,\* Zoology Department, Oregon State University, Corvallis, OR 97331; Elizabeth G. Boulding, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6.

The potential impact of crab predators in the intertidal was examined by setting up field and laboratory experiments at the University of Washington, Friday Harbor Laboratories. A range of sizes of the grazing snail, *Littorina sitkana* was offered to similar sized *Hemigrapsus nudus*, *Lophopanopeus bellus*, *Cancer oregonensis* and *Cancer productus*. Vertical intertidal distributions of snails and crabs were plotted for three beaches varying in wave exposure. Tethered snails were set out at various tidal levels on the same three beaches and the number of live, peeled and crushed snails noted. Peeled shells, consisting of intact columellas with attached whorl remnants, are diagnostic of crab predation.

The ubiquitous shore crabs, *Hemigrapsus nudus* and *Hemigrapsus oregonensis*, coexist with *Littorina sitkana* in the midtidal zone. These crabs will selectively feed on small snails (<7 mm in length), but are ineffective predators when compared to the other three primarily subtidal species. Even though *Lophopanopeus bellus* and *Cancer oregonensis* are small crabs (<40 mm carapace width) they are voracious predators on *Littorina sitkana* up to 12 mm in length.

The red rock crab, *Cancer productus*, attains an adult size of over 150 mm in carapace width and has powerful claws adapted for crushing barnacles, bivalves and gastropods. Even the largest *Littorina sitkana* (17 mm in length) are susceptible to *Cancer productus*. This mobile predator moves up the shore with the incoming tide. Predation rates on tethered snails were as high as 35% and 60% per day at the 4 ft. tidal level at the two most sheltered sites. This study, together with work done by Carlos Robles, suggests that *Cancer productus* is a keystone predator in structuring sheltered intertidal communities.

**HOW DOES LARVAL REARING TEMPERATURE AFFECT SHELL GROWTH AND SURVIVAL OF JUVENILE *CRASSOSTREA GIGAS*?** Kerry M. Zimmerman\* and D. Thompson, Washington Department of Fisheries, Point Whitney Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, WA 98376.

The Washington Department of Fisheries (WDF) is investigating the potential for using oyster larvae to enhance oyster popu-

lations on public tidelands. Oyster larvae are usually reared at high temperatures ( $\geq 27^{\circ}\text{C}$ ), then set and reared as juveniles at cooler temperatures ( $\leq 22^{\circ}\text{C}$ ). Past field experiments have shown that such larvae set successfully in the field only when ambient water temperatures are greater than  $20^{\circ}\text{C}$ . We conducted a series of experiments to see how rearing temperature affects the setting of larvae, and the survival and shell growth of juveniles.

Larvae of *Crassostrea gigas* were reared at  $22^{\circ}\text{C}$  and  $27^{\circ}\text{C}$ . Once larvae became fully eyed and exhibited pedal activity, we

exposed the larvae to  $1.0 \times 10^{-4}$  M L-DOPA (Coast Oyster Co.) to induce searching behavior and metamorphosis. Following metamorphosis, we maintained juveniles at  $22^{\circ}\text{C}$  and  $18^{\circ}\text{C}$ . The percentage of larvae induced to metamorphose and their shell lengths were determined 6 days (d) after exposure to L-DOPA. We also measured shell lengths and determined survivorship of the juveniles 13 d following exposure to L-DOPA. Preliminary data indicate that juveniles reared at  $22^{\circ}\text{C}$  as larvae have greater survival and shell growth than juveniles reared at  $27^{\circ}\text{C}$  as larvae.

**ABSTRACTS OF TECHNICAL PAPERS**

*Presented at the International Zebra Mussel Research Conference*

Columbus, Ohio

December 5 — 7, 1990

## INTERNATIONAL ZEBRA MUSSEL RESEARCH CONFERENCE

The Ohio Sea Grant College Program and The Ohio State University are very pleased to welcome you to our campus and host this outstanding conference for the six Sea Grant Programs in the Great Lakes Sea Grant Network. We have selected this late fall date to allow research scientists to present their results from the current sampling year and to foster cooperation and collaboration on research projects planned for next year.

Lake Erie is one of Ohio's greatest natural resources and the Great Lakes represent one of the region's greatest natural resources. Every day over 11 million people get their drinking water from Lake Erie and many more receive electricity from power plants located on its shoreline. Our ability to use Lake Erie water for these purposes is now hampered by zebra mussels which are also threatening the recreation/tourism industry and a naturally reproducing walleye population with an annual economic value in excess of \$500 million. The Ohio sport harvest of walleye has grown from 112,000 in 1976 to the point that we frequently approach or surpass five million. A rapidly growing charter fishing industry (34 captains in 1976 to over 1200 last year) has bolstered this fishery, but it too is being adversely affected by zebra mussels.

Scientists believe zebra mussels were introduced into the Great Lakes in Lake St. Clair in 1985 or 1986. The first official siting in Lake Erie appears to have been by Dr. David Garton at Stone Laboratory in October 1988. By the autumn of 1989, one year later, densities in the western basin had reached 70,000 per square meter. This year densities almost ten times that large have been reported at water intakes.

At this point in time, the future seems uncertain. While some predict that zebra mussels will not be as much of a problem in the other Great Lakes as they are in Lake Erie because biologically these lakes are not as productive, as the mussels move inland, the impact in our inland lakes and streams could be much more severe.

The research results presented in the abstracts contained in this document represent a huge step forward in our understanding of the entire zebra mussel problem. I believe it is important that we recognize that these important results have been obtained by outstanding and dedicated scientists most of whom have operated with severe budget constraints. Next year's conference will be hosted by New York Sea Grant College Program, and, with funds recently appropriated by the U.S. Congress, I anticipate even more significant advances to address this issue.

Sincerely,

*Jeffrey M. Reutter*, Ph.D.  
Director

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## KEYNOTE PRESENTATION

**PRESENT STATUS OF *DREISSENA POLYMORPHA* IN CENTRAL EUROPE.** Anna Stanczykowska-Piotrowka, Department of Ecology and Environment Protection, Institute of Biology: Agricultural-Pedagogical University, 08-110 Siedlce, B. Prusa 12.

Information on the ecology of *Dreissena polymorpha* Pall. has been collected in my own long-term studies and from the literature. *Dreissena polymorpha* has spread throughout the freshwater lakes in Europe during the past 200 years. This process is still occurring, as for instance in the sixties and seventies in Swiss and Italian lakes. The character of occurrence, population density, biomass and production are different in various ecosystems and changes with time, as seen in lakes in North-Eastern Poland, in the Firth of Szczecin (Poland), Balaton Lake (Hungary) and in Uchinsk Man-Made Lake (USSR). *Dreissena polymorpha* shows several different patterns of population dynamics, due for many possible reasons. As a consequence, the role of zebra mussels in food chains in different freshwaters and in different years varies widely.

**SEASONAL PATTERNS OF REPRODUCTION AND LARVAL ABUNDANCE OF *DREISSENA* IN WESTERN LAKE ERIE: WHAT A DIFFERENCE A YEAR MAKES.** David W. Garton and Wendell R. Hagg, The Ohio State University, Department of Zoology, 1735 Neil Avenue, Columbus, OH 43210.

Seasonal patterns of gametogenesis, planktonic veliger abundance and larval settlement were monitored at Stone Laboratory in western Lake Erie during 1989 and 1990. Samples of adult *Dreissena* were collected biweekly and fixed for histological examination of gonad maturation. Veliger larvae were collected in weekly quantitative vertical plankton hauls (net mesh 45 and 64  $\mu\text{m}$ ). Recently settled larvae were enumerated on glass slides using a stereomicroscope and settling plates were examined every five to seven days during summer months.

For both years veliger larvae were present in plankton samples from mid-June to mid-October. In 1989, abundance of veligers in the plankton and settling on glass plates exhibited a distinct bimodal pattern, with peak abundances occurring in late July and late August. However, histological analyses of gonad condition revealed highly synchronous spawning activity in the local population, with spawning occurring only once, in late August. These temporal differences between histological data and abundance of veligers in the plankton can be explained by asynchronous spawning among disjunct populations of *Dreissena* in western Lake Erie, probably resulting from heterogeneous hydrological environments.

In contrast to the bimodal pattern in 1989, in 1990 there was only a single peak in planktonic veliger abundance and larval set-

ling at Stone Laboratory. Peak veliger abundance occurred in late July, with peak larval settling occurring several weeks later in August. Overall, fewer larvae settled during the summer of 1990 than 1989 (17,390  $\text{m}^{-2}$  versus 31,600  $\text{m}^{-2}$ , respectively), although peak settling intensities were nearly identical (1,650 versus 1,890 larvae  $\text{day}^{-1} \text{m}^{-2}$ , respectively). Histological analyses of gametic development for 1990 samples are not yet complete, therefore it is not known when the local population spawned, or if spawning was synchronous as in 1989.

Several factors may explain the difference in larval abundance and settling patterns between 1989 and 1990. Peak summer temperatures were 5°C cooler in 1989 than 1990 (25 versus 30°C, respectively), and many cold fronts moved across Lake Erie during the summer of 1990, resulting in higher rainfall, storm surges and sediment inputs than during summer of 1989. The density of resident *Dreissena* may also have been a factor. Initial density of *Dreissena* in early 1989 was approximately 400  $\text{m}^{-2}$ , which rose to over 30,000  $\text{m}^{-2}$  following recruitment. Differences in adult density (and concomitant grazing on phytoplankton), coupled with less-than-optimum environmental conditions, are possible mechanisms responsible for annual variation in spawning and recruitment of *Dreissena* in western Lake Erie.

**DISTRIBUTION OF ZEBRA MUSSEL VELIGERS IN EASTERN LAKE ERIE DURING THE FIRST YEAR OF COLONIZATION.** Howard P. Riessen, T. A. Ferro, and R. A. Kamman, SUNY College at Buffalo, Department of Biology, 1300 Elmwood Avenue, Buffalo, NY 14222.

We investigated the seasonal and spatial distribution of zebra mussel veligers during the first year of colonization of this species (1989–1990) in the far eastern end of Lake Erie (Silver Creek, N.Y. to Buffalo, N.Y.). Veligers were first observed in this region of the lake in mid-August 1989 in very low densities (70/ $\text{m}^3$ ). By mid-September densities increased to 300–3,000/ $\text{m}^3$ , with higher densities at western stations (closer to the population source) and lower densities to the east. Densities declined rapidly during October, and veligers were absent from the water column by early November.

During 1990, veliger larvae were absent during May and June, but densities increased rapidly during July and August, reaching peaks at some stations in excess of 100,000/ $\text{m}^3$  in August. Densities decreased very rapidly during September to less than 500/ $\text{m}^3$  by the end of the month. Inshore stations (9–10 meters deep) had considerably higher densities than offshore stations (15–22 meters deep) in the lake. The delay in veliger production until early July in 1990 appears to be related to the age structure of the population. The first mussels settled in this region of Lake Erie during mid to late summer 1989, and probably did not mature and begin producing veligers until mid-summer 1990 at about one year of age. Veliger larvae were distributed fairly evenly throughout most of the water column, with somewhat lower densities near the

surface and higher densities near the bottom. During the first year of colonization of zebra mussels in this region of Lake Erie, veliger densities increased by one to two orders of magnitude. This rate of increase is similar to that experienced during the first year of invasion in the western basin of the lake.

**SPATIAL DISTRIBUTION AND DISPERSAL MECHANISMS OF ZEBRA MUSSELS IN THE GREAT LAKES BASIN.** Ronald W. Griffiths, Ministry of the Environment, 985 Adelaide Street South, London, Ontario N6E 1V3.

*Dreissena polymorpha* (Pallus), a small mussel common throughout most of Europe, was discovered in June of 1988 in the southwestern corner of Lake St. Clair. Length frequency analyses of populations from the lower Great Lakes and review of historical benthic studies suggest that the mussel was introduced to Lake St. Clair in late 1986. Following the 1989 reproductive season, *Dreissena* was found from the lower half of the St. Clair River, through Lake St. Clair, the Detroit River, Lake Erie, the Welland Canal, the Niagara River and into the western basin of Lake Ontario. Isolated populations were also observed in the St. Lawrence River, three harbors in Lake Huron and Lake Superior and inland lakes of Ohio, Pennsylvania and New York. The rapid dispersal of this organism is a result of its high fecundity, pelagic larval stage, bysso-pelagic drifting ability of juveniles and human activities associated with commercial shipping, fishing and boating (research and pleasure). Virtually any waterbody that can be reached by boaters and fishermen within a few days travel of Lake Erie are at risk of invasion from this nuisance species.

**COMPARISON OF THE ZEBRA MUSSEL INVASION IN WESTERN LAKE ERIE, NORTH AMERICA AND LAKE BALATON, HUNGARY.** Don W. Schloesser, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI 48105; William P. Kovalak, Detroit Edison Company, 2000 Second Avenue, Detroit, MI 48226-1279; Thomas F. Nalepa, Great Lakes Environmental Research Lab, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105.

The zebra mussel, *Dreissena polymorpha*, was first discovered in the Great Lakes in 1988. By the fall of 1989, the mussel was recognized as a potential ecological and economic disaster for North America. Extensive reviews of the European literature, where over 1500 articles have been published concerning zebra mussels, revealed important information about the impacts of the zebra mussel on water withdrawal. However, no case histories of zebra mussel invasion in Europe seemed to parallel the speed and magnitude with which the mussels invaded North America. In addition, the observable ecological impacts in Lake Erie were not found in the European literature, except for the work of Olga Sebestyen. This work partially summarizes the invasion of zebra mussels in the 1930s in Lake Balaton, Hungary. Lake Balaton is central Europe's largest lake and has many characteristics similar

to western Lake Erie. The Lake Balaton experience confirms that the speed and magnitude of the invasion of zebra mussels into North America is uncommon but not unique. Based on the Lake Balaton and other European experiences, the extremely high densities of zebra mussels in western Lake Erie likely will begin to decline. However, the rate of decline will be difficult to predict and, therefore, continued research and monitoring will be required to document and manage; as much as practical, the impacts of the zebra mussels on water withdrawal and indigenous species in western Lake Erie.

**TEMPORAL CHANGES IN ZEBRA MUSSEL (*DREISSENA POLYMORPHA* PALL.) VELIGER DENSITIES AND VELIGER SETTLING RATES IN WESTERN LAKE ERIE NEAR MAUMEE BAY.** Peter C. Fraleigh and Paul L. Klerks, University of Toledo, Biology Department, 2801 W. Bancroft Street, Toledo, OH 43606; Robert C. Stevenson, City of Toledo, Division of Water, Toledo, OH.

From May 1, 1990, weekly sampling for zebra mussel veligers has been done in western Lake Erie near the Toledo Water Intake (41°42.0' N, 83°15.6' W, water depth—seven meters) and from the surge well at the Toledo Low Service Pump Station, on the shore at the end of a three-mile (5-km), 108-in (2.7-meters) diameter pipe from the water intake. Planktonic veligers were collected by vertical haul to the surface, from 5 meters at the intake and from 3 to 5 meters in the surge well, with an 80- $\mu$ m mesh Wisconsin style plankton net. Settled veligers were collected near the water intake on 1  $\times$  3 inch glass slides mounted on a rack in the hole of a cinder block. Planktonic veligers were not found on May 25, and first appeared on May 31 (100/L) when water temperature was 16.7°C. Between May 31 and August 28, the geometric mean density was 72/L, and veliger densities ranged from lows of around 20/L on July 2, 16 and August 9 to highs of 250/L on July 24 and August 2, 150/L on August 21, and 140/L on June 12. Maximum water temperature during this period was 24°C. Since August 28, veliger densities have declined. Geometric mean veliger density in the surge well was 22 percent that in the lake (16/L). Feeding by hydra and filtering by mussels attached in the pipe may have been contributory. It does not appear that the reduction in veliger densities in the pipe is due to settling of the veligers, since the loss also occurred prior to the period that the mussels started settling in the lake. Settled veligers were first found on July 16 (0.01/m<sup>2</sup>/week, water temperature 21°C). Between July 16 and September 20, when the lake was warmest for the year, the geometric mean settling rate was 1700/m<sup>2</sup>/week. Thus, in Lake Erie near Maumee Bay, settling with attachment began about 45 days after planktonic veligers were first observed. Most settling occurred when water temperatures were at maximum values for the year. In October, settling continued at a lower rate as the water temperature declined.

**TEMPERATURE-DEPENDENT METABOLISM OF ZEBRA MUSSELS: SEASONAL AND SHORT-TERM ACCLIMATION EXPERIMENTS.** David W. Garton and Ann M. Stoeckmann, The Ohio State University, Department of Zoology, 1735 Neil Avenue, Columbus, OH 43210.

The metabolic response of zebra mussels, *Dreissena polymorpha*, to seasonal change of ambient water temperature was measured weekly between June 26 and October 16, 1990. Oxygen consumption was measured using a Gilson Differential Respirometer and nitrogen excretion was measured simultaneously by spectrophotometric determination of ammonia (phenol-hypochlorite method). Mussels ( $N = 33-38$ ) were separated from their substrate by gently severing byssal threads and acclimated in the laboratory for 24 hours at ambient temperature prior to measuring metabolic rates. Metabolic rates were standardized to the overall mean dry weight, 19.6 mg (range 1.0–64.6 mg,  $N = 427$ ) using analysis of covariance. Oxygen consumption ( $VO_2$ ) was expressed as  $\mu\text{l O}_2 \text{ hr}^{-1}$  per standard mussel at STP; ammonia excretion ( $VNH_4$ ) as nM ammonia  $\text{hr}^{-1}$  per standard mussel. All work was conducted by F. T. Stone Laboratory, located in western Lake Erie. Ambient water temperature ranged from 15–25°C.

Both  $VO_2$  and  $VNH_4$  showed similar seasonal patterns, with metabolic rate peaking on July 5, and remaining essentially unchanged during the remainder of the summer and fall. The peak in metabolic rate occurred two weeks prior to the peak in veliger abundance in plankton samples, indicating a possible correlation with mussel spawning activity and metabolic rates. Analysis of histological samples will confirm this relationship. Reduced phytoplankton populations following a mid-July clear-water phase may also explain reduced metabolic rates after July 5. This hypothesis is supported by the observation that mussels collected after July 5 produced less feces in the laboratory than mussels collected prior to this date. Although there was a statistically significant positive relationship between metabolism and temperature, the correlation coefficients were quite small ( $VO_2$ ,  $r = 0.117$ ,  $p < .018$ ;  $VNH_4$ ,  $r = 0.262$ ,  $p < .0001$ ). In general, zebra mussel metabolic rates were constant across seasonal temperature gradients.

Short-term acclimation of  $VO_2$  to rapid increases in temperature were also examined.  $VO_2$  was measured prior, immediately following, and two and five days post-transfer to 31°C (long-term acclimation above this temperature was lethal). Oxygen consumption increased three-fold with the increase in temperature, and showed no decline (acclimation) toward pre-transfer levels by the fifth day post-transfer. Although *Dreissena* can maintain constant metabolic rates across seasonal water temperature gradients between 15–25°C, it loses this ability dramatically above 30°C.

**POPULATION DYNAMICS OF *DREISSENA POLYMORPHA* IN THE GREAT LAKES: PREDICTIONS BASED ON THE EUROPEAN EXPERIENCE.** Charles W. Ram-

charan, University of Wisconsin, Department of Zoology, Birge Hall, 430 Lincoln Drive, Madison, WI 53706; Diana K. Padilla and Stanley I. Dodson, University of Wisconsin, Department of Zoology, Birge Hall, Madison, WI 53706.

Populations of *Dreissena polymorpha* exhibit a wide range of population dynamics in European lakes. We have found that in some lakes abundance of *Dreissena* is stable over time periods of 10–30 years, while in other lakes populations show interannual fluctuations of more than an order of magnitude. If populations of *Dreissena* in the Great Lakes show the latter boom-and-bust cycle, the problems caused by this mussel, such as biofouling of water intake pipes, ships' hulls, and fish spawning beds, and consumption of algal productivity, would only be severe every few years during population peaks. Long-term population sizes may be lower than are now found in Lakes Erie and St. Clair. Predictions of the ecological role of *Dreissena* in the Great Lakes would have to consider the range over which populations might fluctuate from year to year, and the mechanisms that cause fluctuations.

We construct a model to predict the potential population dynamics in *Dreissena* in the Great Lakes using data collected from published European studies, using multivariate techniques. We first divide European lakes into two categories (stable and unstable), based on interannual variations in the abundance of *Dreissena*. We then use data on the morphometric, physical, and chemical environments of these lakes in Discriminant Function Analyses to find a multivariate model that best differentiates these two lake types. Unstable populations of *Dreissena* tend to be found in deep lakes that have high flushing rates and small surface areas. Larger, shallower basins with low flushing rates tend to have more stable populations.

**COLONIZATION OF SOFT SUBSTRATA BY ZEBRA MUSSELS: ROLE OF AND COST TO NATIVE BIVALVES.**

R. Douglas Hunter and John F. Bailey, Oakland University, Biological Sciences, Rochester, MI 48309-4401.

Sites in the western, central and eastern part of southern Lake St. Clair were surveyed, using SCUBA in late September, 1990, for zebra mussels and hard substrata. Like much of the lake, the bottom in this region mostly consists of silty clay overlain by a thin layer of detritus. From west to east, zebra mussel density increased: west, 213 individuals/m<sup>2</sup> (26.1 g/m<sup>2</sup> live mass, shell + soft tissues), central, 1907/m<sup>2</sup> (402.1 g/m<sup>2</sup>); east, 12,726/m<sup>2</sup> (845.8 g/m<sup>2</sup>). Each site was significantly different from the other two sites in terms of zebra mussel biomass/m<sup>2</sup> (one-factor ANOVA,  $P < 0.0001$ ; Scheffe's F-test,  $P < 0.001$ ). Both abundance and biomass of live native bivalves decreased from west to east: west, 2.3/m<sup>2</sup> (129.5 g/m<sup>2</sup>); central, 1.3/m<sup>2</sup> (73.4 g/m<sup>2</sup>), east, 0.1/m<sup>2</sup> (16.6 g/m<sup>2</sup>). Solid substrata in this area are relatively scarce and consist almost entirely of live or dead native bivalves. Such surfaces are intensely settled by zebra mussels, so much so that at the east site, a mat of zebra mussels extending from the

more heavily colonized native bivalve shells was observed in several of the more densely settled sample plots. Among the three sites there was a strong negative correlation between zebra mussel biomass and number of live native bivalves ( $r = -1.0$ ,  $p < 0.01$ ,  $N = 3$ ). The ratio of live to dead native bivalves decreased from west to east (west = 25:1; central = 0.9:1; east = 0.02:1). Many of the more heavily colonized native bivalves (whether live or dead) showed damage to the posterior valve edges, which is where zebra mussel accumulations were heaviest. The general condition of zebra mussels also appeared to worsen from west to east. At the west site, the zebra mussel clusters were relatively uncrowded (monolayer) with few empty shells and uneroded periostraca, whereas at the east site, many individuals were dead, having been on the bottom of extensive colonies. Further, the shells of live animals often had sizable areas of periostracum eroded away. The size/frequency distribution of zebra mussels at the three sites suggested two generations were present: young of the year and one-year-old individuals. There were few individuals over 20 mm shell length. At the west site there was an obvious lack of newly settled spat (i.e., <3 mm shell length). At the other two sites such spat comprised the most abundant size classes.

In spite of a very extensive area of soft bottom, southern Lake St. Clair supports a moderately high density of zebra mussels; nearly 13,000/m<sup>2</sup> at some sites. In the areas sampled, native bivalves (both live and empty shells) were the most common hard surface on which to settle, and were negatively correlated with zebra mussel density. Direct field observations as well as the data presented above, strongly suggest that the community of native bivalves is being severely reduced as a direct result of zebra mussel colonization.

**GROWTH RATES OF *DREISSENA POLYMORPHA* IN THE ST. CLAIR RIVER AND LAKES ST. CLAIR, ERIE AND ONTARIO FROM JUNE TO NOVEMBER 1990.** Susan Jerine Nichols, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI 48105; Angela M. Bitterman, Michigan Dept. of Natural Resources, 2463 Otter, Warren, MI 48092; Fleur Ely, Ontario Hydro, 700 University Avenue, Toronto, Ontario M5G 1X6.

In June, 1990, we began a study of growth rates of zebra mussels (*Dreissena polymorpha*) at 6 sites in the Great Lakes. The mussels were placed in individual compartments in plexiglass cages, which were deployed at Marine City in the St. Clair River, Clinton and Thames River areas of Lake St. Clair and at the Lakeview, Monroe and Nanticoke power plants in Lake Erie and the Lakeview power plant in Lake Ontario. Shell length and chlorophyll, current velocity, density and type of planktonic algae, dissolved calcium, Secchi depth, total and particulate carbon, and water temperature were measured at either biweekly (Lake St. Clair) or weekly (all other sites) intervals. This long-term study is intended to provide data to enable us to determine changes in

growth rates across environmental gradients. It should be possible to develop a model from this information that can be used to predict the ultimate spread and overall density of zebra mussels across the country.

Preliminary data analysis indicates that growth rates varied considerably by site, date and length cohort. In general, growth was highest at Monroe, with an average relative growth rate each week of about 53%, and lowest at Nanticoke with less than 10% weekly growth. The Thames River and Marine City were similar in growth rates (30 to 35%) as were Clinton River and Lakeview sites (20 to 25%). Growth rates at all sites varied significantly from week to week. For example, at the Thames River site, growth rates of the 2 mm size cohort ranged from 7 to 100% over a 6 week period. The growth rate was consistently greater in the smaller mussels (<4 mm) and gradually declined as mussels grew larger. The relative small size of the cage compartment (13 mm in diameter) restricted length of the shell as the mussels grew larger. However, growth in width, height and weight were not constrained by the compartment size. Mussels were replaced with smaller individuals or placed in larger compartments when they reached 12 mm.

At this time, the most critical environmental parameter for predicting growth is total carbon. We have measured both particulate and dissolved carbon, but our work indicates that dissolved organic carbon is not used by zebra mussels. Lower autumn and winter water temperatures are expected to be reflected in growth rates. Chlorophyll and algal data are very inconsistent and not very useful.

**PLANKTON DIATOMS IN HATCHERY BAY, WESTERN LAKE ERIE, BEFORE AND AFTER THE INVASION OF THE ZEBRA MUSSEL.** Ruth E. Holland Beeton, University of Michigan, Atmospheric, Oceanic & Space Science, 2761 Oakcleft Court, Ann Arbor, MI 48103.

Plankton diatoms from Hatchery Bay in the Bass Island region, western Lake Erie, were compared for April–September of 1984, 1985 and 1986, prior to the invasion of *Dreissena polymorpha* Pall. and 1990, when it had become firmly established. The mean value of total frustules/ml in 1990 declined over 81% from mean values in the 1980s. The abundance of all planktonic diatoms which were major species in the '80s had decreased by 1990, except for *Aulacoseira granulata* (Ehrenb.) Simonsen, whose numbers increased slightly. Epiphytic species, absent or occasional in the 1980s, sometimes made up over half the total number of frustules in the 1990 samples; this was especially true in summer for *Cocconeis placentula* var. *euglypta* (Ehrenb.) Cleve which accounted for up to 66% of the abundance of all species.

**ALGAL HERBIVORY BY THE ZEBRA MUSSEL: FATE OF ALGAE IN FECES AND PSEUDOFECES.** Rex L. Lowe, LouAnne Reich, and Jim Sierra, Bowling Green State Univer-

sity, Department of Biological Sciences, Bowling Green, OH 43403.

This project was designed to determine the fate (survivorship and growth) of suspended algae in Lake Erie that have been filtered by zebra mussels. Mussels were collected near Catawba Island and transferred to the laboratory. Feeding experiments were conducted in the laboratory at two week intervals from June through September, 1990. Mussels were exposed to fresh Lake Erie water and allowed to filter for four to eight hours. Five pseudofecal pellets were collected during each feeding bout. Each was washed serially in filtered Lake Erie water and then transferred into 50 ml flasks containing filtered Lake Erie water and incubated for two weeks in a growth chamber. Two fecal pellets were collected and cultured similarly during the feeding bouts. Following incubation, pellet cultures were preserved, homogenized and quantified in a nanoplankton counting chamber at 400 $\times$  magnification. Survival of algae following gut passage in fecal pellets is minimal. Some benthic algae apparently survive but other fecal pellets contained no viable algal cells. Algal growth from pseudofeces was dominated by green algae in June with *Microspora* dominating the algal community. In July, green algae diminished in importance and diatoms (species of *Navicula* and *Nitzschia*) increased in relative abundance. In late August and September blue-green algae, *Anabaena* and *Oscillatoria* codominated with green algae and diatoms. In general benthic algae appear to be able to escape pseudofecal pelletization better than typically planktonic species. This fact in concert with increased water transparency may lead to a shift toward increased importance of benthic algae in Lake Erie.

#### INTERACTIONS BETWEEN ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) AND PELAGIC COMMUNITIES.

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While zebra mussels might interact with pelagic communities via altering availability of dissolved oxygen, excretion of nitrogen and phosphorus, and sedimentation of silt and algae through the formation of pseudofeces, an obvious way in which they might impact pelagic communities would be through removal of phytoplankton that otherwise would be eaten by zooplankton. This activity might also be reflected in increased clarity of the water which would favor growth of algae, including benthic forms, at depths where zebra mussels may be found. Such variations in water clarity were found in 1988 in the western basin of Lake Erie where zebra mussels were present, as compared to 1989 when they were abundant. Maximum Secchi transparency in 1988 was 3.4 meters (26 June) at an offshore station, whereas the 1989 maximum transparency at the same station was over 6.5 on 4 July. Estimation of grazing rates by *Daphnia galeata mendotae* and *D. retrocurva*, however suggests that these differences could be ex-

plained by plankton grazing alone, which at 200 ml L<sup>-1</sup>d<sup>-1</sup> was almost 4 times the grazing rate during the same period in 1988. This was a result of both higher abundance of *Daphnia* spp. and a larger average size of these taxa. The impact of zebra mussels on algal abundance during this period is unknown, but the high grazing rates of freshwater Cladocera generates a condition unlike that found in many marine environments where zooplankton were found to have a relatively small impact on algal abundance compared to bivalves. Changes in nutrient availability, N:P ratios, etc. can also affect the relative abundance and species composition of the phytoplankton.

To determine the impact of zebra mussel grazing, we propose to model the hydrodynamics of mixing in the western basin of Lake Erie as it pertains to the delivery of algae to the benthos. The dimensions of the benthic boundary layer at various depths where zebra mussels may be found will determine the rate at which algae from the euphotic zone are mixed down to the zebra mussels, and whether the final rate of intake is turbulent or is limited by algal sinking rates within the boundary layer. This, along with distribution and abundance in the western basin, size-specific grazing rates, and size-frequencies will enable us to compare the relative impact of zebra mussels on the phytoplankton in the pelagic food web of Lake Erie.

**PHYSICO-CHEMICAL EFFECT OF ZEBRA MUSSEL, *DREISSENA POLYMORPHA*, ON SPAWNING HABITAT AND EGG HATCHABILITY OF WALLEYE, *STIZOSTEDION VITREUM VITREUM*.** **Bruce Vondracek and Jeanette Buenger,** The Ohio State University, Ohio Cooperative Fish & Wildlife, 1735 Neil Avenue, Columbus, OH 43210; **Ruth A. Pontius,** The Ohio State University, Department of Zoology, 1735 Neil Avenue, Columbus, OH 43210.

Freshly fertilized walleye, *Stizostedion vitreum vitreum* eggs (2,668  $\pm$  131) were placed into 38 liter flow-through aquaria containing three densities of zebra mussel, *Dreissena polymorpha* (0, 2,000 and 20,000/m<sup>2</sup>). Seven replicates were used for each treatment. In addition, two replicates containing three densities of mussels but without walleye eggs were also monitored. Temperature, dissolved oxygen, pH and ammonia were monitored daily while walleye eggs were incubating.

No eggs hatched. A fungal infection first noted two days after eggs were introduced into the aquaria resulted in the death of most eggs. Twenty days after eggs were introduced approximately 1 cm of silt and organic matter was pumped into each aquarium, the result of a storm. The silt resulted in the death of all remaining eggs.

Several eggs were examined 8 days after eggs were introduced into the aquarium. These eggs appeared to be developing normally; a head, body and tail were visible. Temperature in the aquaria were within 0.5°C of lake water temperature and no differences were noted across treatments. Dissolved oxygen was at

or near saturation in all aquaria with no differences across treatments and did not fall below 8.0 ppm. pH did not differ across treatments and remained constant at 7.9 throughout the experimental period. Ammonia weighed between 0.03 and 0.12 mg/liter, and although more variable than other parameters, treatments were not significantly different on most dates.

**IMPACT OF *DREISSENA* ON FISHERIES POTENTIAL IN LAKES ST. CLAIR AND ERIE.** Ronald Dermott, Fisheries & Oceans Canada—GLLFAS, Great Lakes Lab, P.O. Box 5050, CCIW, Burlington, Ontario L7R 4A6; J. Leslie, J. Fitzsimmons, and V. Cairns, Bayfield Institute, Fisheries and Oceans, P.O. Box 5050, Burlington, Ontario L7R 4A6.

Fisheries & Oceans (Canada) has been examining the biological impact of zebra mussels on fish habitat in Lakes St. Clair and Erie. During spring 1990, we found no reduction in survival of walleye eggs collected from mussel covered spawning shoals in western Lake Erie. The number of walleye eggs laid on heavily encrusted areas was equal to areas having no zebra mussels. Larval fish populations in eastern Lake St. Clair were similar to that during 1983–1984, prior to the invasion of the mussels. In mid-July, there was no difference in mean weight or lipid content of larval gizzard shad in Lake St. Clair as compared to a site in the St. Clair delta without mussels. In early summer, mussel growth averaged 3 mm month<sup>-1</sup> at a site in eastern Lake Erie where the numbers increased from 385 to 54,000 m<sup>-2</sup> during 1990, representing an increase in dry shell biomass of 717 g m<sup>-2</sup>. Total wet biomass excluding *Dreissena* increased from 6 g m<sup>-2</sup> on bare rock to over 124 g m<sup>-2</sup> in the mussel shoals. The majority of this is due to a huge increase in *Gammarus* numbers under the mussels. Although only 14% of the dry weight of *Dreissena* is soft tissue, the meats are high in lipid, averaging 26%. This makes the mussels a potential energy and contaminant source.

**DIRECT SUPPRESSION OF LAKE ERIE ZOOPLANKTON BY ZEBRA MUSSELS (*DREISSENA POLYMORPHA*).** Hugh J. MacIsaac and W. Gary Sprules, University of Toronto, Department of Zoology, Erindale Campus, Mississauga, Ontario L5L 1C6.

We examined the potential of adult zebra mussels (*Dreissena polymorpha*) to suppress co-occurring species of Lake Erie zooplankton with *in situ* experiments conducted in 4 L glass bottles, and in 50 ml laboratory containers. Susceptibility to suppression was related primarily to body size; two small-bodied, common, rotifer species (*Keratella crassa*, *Polarthra* sp.) were highly suppressed (91 and 96%) by filtering mussels, while a larger rotifer species (*Trichocerca multicornis*) and zebra mussel veligers were somewhat less suppressed (80% and 50% respectively). The small cladocerans *Scapholeberis kingi* and *Bosmina longirostris* were not susceptible to predation (<10% suppression); individuals entrained in the inhalant current of feeding mussels evaded the in-

current siphon by initiating rapid escape maneuvers. We propose that the suppressive mechanism was predation and not exploitative competition because populations of zooplankton in food-supplemented cultures exhibited similar patterns of suppression as those incubated at ambient food levels. In addition, rotifers were observed entrained in the inhalant current of filtering mussels, and loricae (*K. crassa*) were found in dissected mussel digestive tracts but not in pseudofeces following short-term incubations. Our data, while preliminary, provide support for Mikheev's (1967) contention that adult mussels can consume large particles, and suggest that littoral populations of adult mussels may exert strong selective pressure on the size-structure of near-shore zooplankton communities.

**THE ROLE OF ZEBRA MUSSELS IN CONTAMINANT CYCLING IN THE GREAT LAKES.** Peter F. Landrum, Great Lakes Environmental Research Lab, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105; Duane C. Gossiaux, Great Lakes Environmental Research Lab, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105-1593; Susan W. Fisher, The Ohio State University, Department of Entomology, 1735 Neil Avenue, Columbus, OH 43210; Kathleen A. Bruner, The Ohio State University, Department of Zoology, 1735 Neil Avenue, Columbus, OH 43210.

Toxic contaminants, one of the major Great Lakes' problems, exhibit high levels in shallow nearshore areas. Because the zebra mussel will primarily populate these shallower regions of the Great Lakes, they will likely be exposed to the relatively high contaminant concentrations. Further, because of the high biomass in the areas populated by zebra mussels, the organism could have a significant impact on the contaminant cycling. Both toxicokinetic and physiological measures were made to examine the movement and importance of contaminants passing through this new portion of the Great Lakes' food web. The toxicokinetic studies included measures of the uptake and elimination of selected polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH) congeners and DDT. The toxicokinetic parameters were evaluated with respect to the respiration of the organism, the efficiency of oxygen accumulation and the size of the mussels.

The uptake clearance of the most non-polar compound 2,4,5,2',4',5'-hexachlorobiphenyl (HCBP) is 800 ml/g/h at 20°C. This implies a filtration rate of at least 800 ml/g/h if the efficiency of uptake from the water is 100%. Comparing this measurement with the clearance of oxygen (33.6 ml/g/h) implies that the relative efficiency for oxygen accumulation is approximately 4%. The  $q_{10}$  for the organism is 2.1; thus, the accumulation rate will drop by a factor of two for each 10°C change in temperature.

The uptake is only half of the characteristics necessary for understanding net accumulation. The elimination rate coefficient for HCBP is 0.004 per hour and results in a half life for HCBP of 173

hours (7.3 days). Combining the two measures the resultant bio-concentration factor (BCF) of 177,956 can be calculated. Thus, these organisms can be expected to concentrate contaminants about 100 times more than would be estimated for fish. The BCF observed from laboratory studies depends on the hydrophobicity of the contaminant as represented by the log of the octanol:water partition coefficient ( $\log K_{ow}$ ) and the environmental temperature [ $\log BCF = 0.437 (0.015) \log K_{ow} - 0.070 (0.006) \text{Temperature} + 3.696 (0.169)$ ,  $r^2 = 0.996$ ,  $n = 6$ ]. This bioaccumulation is for accumulation from water only. Since most of the important contaminants reside primarily on particles, these filter feeders are expected to have large additional contributions from ingesting contaminated particles. The large BCF for these animals suggests that organisms preying on zebra mussels will be exposed to large contaminant concentrations. Further, because of the large filtration rates suggested from the kinetics, the organisms are expected to effect water clarity and particle transport within the local areas of high population. Thus, these organisms should affect the cycling and transport of contaminants in the Great Lakes.

**EFFECTIVENESS OF MOLLUSCICIDES, ANTIATTACHMENT AND ANTIBIOFOULING AGENTS AGAINST THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*: RESULTS OF LABORATORY AND FIELD STUDIES.** Susan W. Fisher, The Ohio State University, Department of Entomology, 1735 Neil Avenue, Columbus, OH 43210.

The zebra mussel testing center was developed at The Ohio State University to evaluate candidate chemicals for controlling the zebra mussel in both laboratory and field settings, to explore the development of specific compounds which inhibit byssal thread secretion and to serve as a liaison between EPA and industry in developing standardized protocols for zebra mussel testing. A variety of molluscicidal and molluscistatic products were evaluated against the zebra mussel in laboratory and field studies. Of particular interest is the finding that environmentally innocuous chemicals can kill adult zebra mussels in short periods of time at concentrations averaging 150 ppm. These chemicals had no adverse effects on two nontarget organisms, the fish *Gambusia affinis* and snails belonging to *Helisoma* spp. These chemicals proved to be an effective molluscicide under a variety of environmental conditions. Preliminary investigations into a mode of action in the zebra mussel indicate that they work by destroying gill membranes thereby disrupting respiration. Studies aimed at discerning why zebra mussels are apparently more sensitive to these chemicals than other aquatic species are now underway. They will be tested against zebra mussels in field trials at Lake Erie during the summer of 1991.

**CONTROL OF BIOADHESION BY THE ZEBRA MUSSEL.** Robert E. Baier, State University of New York, Department of Biophysical Sciences, Buffalo, NY 14214.

The zebra mussel, like its marine counterpart macrofouling organisms, can rapidly attach to and metamorphose upon solid substrata of amazing diversity. Differential persistence of this attachment, especially in the presence of mechanical challenges like hydrodynamic shear, for different solid substrata represents a potentially controllable, nonpolluting, fouling-suppression strategy. Our investigations utilize zebra mussel adults harvested from Lake Erie and maintained in (slowly reproducing) fresh water laboratory colonies, together with surface-quality-controlled and carefully characterized test panels in both laboratory and field settings. A major and primary goal is to identify the compositional and configurational features of the exuded mussel adhesive substance that attaches the byssus discs to the substrata. A related, secondary goal is to confirm prior observations made with maritime mussels, barnacles, tubeworms, hydroids, encrusting bryozoa and algae that adjustment of a material's "critical surface tension" to the bioadhesive zone between 20 and 30 dynes/cm will also minimize retention strength of attached zebra mussels.

Regarding the first goal, spontaneous re-attachment of zebra mussels to the optically smooth faces of germanium internal reflection prisms has allowed identification of the in-place bioadhesive substance as a glycoprotein with a presumptively high content of DOPA (dihydroxyphenylalanine). The comparison of this freshwater "mussel-glue" to that obtained from *Mytilus edulis* and other adhesive organisms is currently being examined by biochemical and histological methods, with generally similar early findings. Demonstration of facile release from surface-controlled substrata is routinely obtained using very thin-film coatings of high-molecular-weight polydimethylsiloxane, functionally equivalent to the medical-grade silicone veneers deliberately put in the insides of "artificial hearts" to suppress their biofouling from blood. Work in progress seeks to extend the field lifetimes of these environmentally and ecologically safe coatings, while also examining compatible nonpolluting alternatives including use of fine-textured polymers and illumination with ultraviolet lamps. Although entire avoidance of chlorine is desired, the use of low-surface-energy "fouling-release" coatings together with special biofilm-removal agents based on surface chemical principles does allow chlorine minimization by resuspending microorganisms into the more susceptible planktonic phase.

Specially designed flow cells have been successfully utilized in both laboratory and field settings for the study of biofouling under known and controlled hydrodynamic conditions, allowing testing of various control coatings and techniques. Equipment for unattended, multiply-manifolded flow cell testing is now being assembled for installation at field sites in New York State likely to have high zebra mussel veliger populations in spring, 1991.

**THE ACUTE AND CHRONIC EFFECTS OF A POLYQUATERNARY AMMONIUM MOLLUSCICIDE POLY[OXETHYLENE (DIMETHYLIMINIO)ETHYLENE-(DI-**

**METHYLIMINIO)ETHYLENE DICHLORIDE] ON *PIMEPHALES PROMELAS* AND *CERIODAPHNIA DUBIA*.** John H. Giltner, Jr., 1509 W. Sixth Avenue, Columbus, OH 43212; Paul C. Baumann, The Ohio State University, U.S. Fish & Wildlife Service, 2021 Coffey Road, 473 Kottman Hall, Columbus, OH 43210.

Various polyquaternary ammonium molluscicides are currently being used to control zebra mussel (*Dreissena polymorpha*). Bioassays were conducted to gauge the acute and chronic effects of one such compound on *Ceriodaphnia dubia* neonates and larvae of the fathead minnow (*Pimephales promelas*). In the forty-eight hour acute bioassays, lethal concentration (LC) 50 s of 218 ppb for the *Ceriodaphnia dubia* and 353 ppb for the *Pimephales promelas* were obtained. In the seven-day bioassays, the test concentrations were reduced, and the organisms were fed liberally. Although there was growth impairment in the *Pimephales promelas* larvae and reproductive impairment in the *Ceriodaphnia dubia*, these chronic effects were less than expected. This decrease in toxicity is due to the cationic properties of this molluscicide and may warrant a change in the methods used to assess the chronic effects of cationic polyelectrolytes.

**APPROACHES TO ZEBRA MUSSEL CONTROL THROUGH INTERVENTION IN REPRODUCTION.** Jeffrey L. Ram, Wayne State University, Department of Physiology, Detroit, MI 48201; F. H. Nichols, U.S. Geological Survey, MS 496, 345 Middlefield Road, Menlo Park, CA 94036.

Control methods for zebra mussels are usually applied at impacted sites using biologically nonspecific methods directed at adults (e.g. chlorination) and often require large capital and labor inputs (e.g. dual water intakes and mechanical removal of mussels). Problems with these methods include lack of biological specificity, corrosive chemicals that may damage the physical plant, and, in the case of drinking water, bad taste, odor and release of biofiltered pollutants. An alternative approach is to intervene in the zebra mussel life cycle at an earlier stage, when they are spawning. Previous research with zebra mussels and related bivalves indicate that spawning probably depends on specific chemical environmental cues and a neurally mediated response pathway. Each point along the chemically activated pathway represents a possible control point at which spawning cues or their inhibitors may be employed to induce spawning at times inappropriate for zebra mussel survival or to block natural spawning.

A model for the control of bivalve spawning is that chemicals released by phytoplankton induce males to spawn. Chemical cues from ripe, spawning males induce females to spawn and female chemical cues activate males, providing positive feedback. The neural-gonad response pathway uses serotonin and prostaglandins as mediators. In support of this model in zebra mussels are observations by Garton (oral communication at 1990 AFS meeting) of a correlation of phytoplankton blooms in Lake Erie

with the appearance of veligers, experiments by Walz (1978), Sprung (1989) and Nichols (unpublished data) of female zebra mussel spawning induced by male gonad extracts, and experiments reported here on induction of zebra mussel spawning by serotonin.

Zebra mussels were injected with serotonin (0.1 ml of either  $10^{-3}$  M or  $10^{-5}$  M) or vehicle (0.1 ml), put into separate culture tubes, and surrounding water was sampled for sperm and eggs within 4 hours. After spawning observations were complete, squash mounts of mussels were prepared to assess sex and reproductive maturity. Serotonin induced spawning in 22/23 of ripe (stage 4) males, whereas 0/10 ripe males responded to vehicle ( $p < 0.001$ , Fisher exact test). In partially ripe males (stage 3) serotonin induced spawning in 2/8 males v. 0/3 control males (not significantly different). 1/6 intermediate immature males (stage 2) responded to serotonin v. 0/7 controls (not significantly different). Among female recipients, eggs were found in water surrounding both control and serotonin-injected animals (e.g. stage 4, 6/16 experimental and 3/7 controls), suggesting perhaps only a mechanical effect of the needle, and, in any case, the quantity of eggs released was always much less than observed with natural zebra mussel spawning. Serotonin injection provides a means of identifying ripe males without the need for dissection. Further experiments are needed to identify triggers for female spawning and the environmental chemical triggers that can induce zebra mussel spawning.

**FIELD PERFORMANCE OF SELECTED COMMERCIAL ANTI-FOULING COATINGS AGAINST THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*.** Fred L. Snyder, The Ohio State University, Ohio Sea Grant College Program, Building 3, Room 12, Camp Perry, Port Clinton, OH 43452; David O. Kelch, The Ohio State University, Ohio Sea Grant College Program, 42110 Russia Road, Elyria, OH 44035; Frank R. Lichtkoppler, The Ohio State University, Ohio Sea Grant College Program, 99 East Erie Street, Painesville, OH 44077.

Four types of commercial anti-fouling coatings used by marine industries were tested against zebra mussels in western Lake Erie. Sets of three test strips made of wood, aluminum and marine fiberglass were suspended in boat harbors at the Toussaint River, Beaver Creek and the Ashtabula River. Each strip was coated with randomly arranged squares of commercial brush-on paints containing copper and tributyltin (TBT), a spray-on TBT engine paint, a commercial non-toxic anti-fouling coating and an unpainted control square. The strips were left in the harbors from July through October, 1990, and were examined weekly. The copper and brush-on TBT paints provided complete, season-long protection against fouling by zebra mussels. The spray-on TBT paint provided complete protection for nine weeks, after which a minimal amount of settlement by mussels occurred. The non-toxic anti-fouling coating offered no reliable protection against settle-

ment, and on the wooden strips was frequently more heavily colonized than the uncoated control squares. While TBT-based spray paints appeared to have limited periods of effectiveness, applications in midsummer, prior to zebra mussel spawning should provide acceptable protection to marine engines.

**ZEBRA MUSSEL INFESTATION AT MONROE POWER PLANT: CHANGES DURING 1990.** William P. Kovalak, G. D. Longton, and R. D. Smithee, Detroit Edison Company, 2000 Second Avenue, Detroit, MI 48226-1279.

Infestation of the inlet canal at Detroit Edison's Monroe Power Plant during 1990 was less than 1989. In August 1989 densities exceeded, 700,000/m<sup>2</sup> whereas in August 1990 densities were only 150,000–20,000/m<sup>2</sup>. Lower densities were due to sloughing of clusters (druses) from vertical surfaces (e.g., sheetpiling) and to lower recruitment attributed to lower water temperatures. Although densities were lower, approximately 100 yd<sup>3</sup> of mussels were hydroblasted from 6000 ft<sup>2</sup> of concrete surface in the Unit 2 screenhouse in September 1990. This greater volume (approximately 2×) was due to greater size of age 1+ individuals.

Service water systems also were fouled by zebra mussels. In September 1990 densities inside 30 inch diameter headers were as high as 200,000/m<sup>2</sup>. Heavy fouling extended to butterfly valves ranging in size from 4 to 30 inches in diameter. Since December 1989, nearly 100 valves have been replaced. Presently, these systems are being chlorinated at 3 ppm to mitigate existing infestation.

**PROBLEMS DURING 1990 AT WATER TREATMENT PLANTS IN SOUTHWESTERN ONTARIO, AND POSSIBLE SOLUTIONS.** Mike Auger, Ministry of the Environment, 985 Adelaide Street South, London, Ontario N6E, 1V3.

This talk will discuss the problems experienced with zebra mussel infestation at water plants, located in Southwestern Ontario. In particular, the problems of continual plugging of the low lift pumping station travelling screens with clusters of zebra mussels during the high water demand period at the Union Water Plant.

Also, the problem of loss of intake capacity (i.e., 50% loss at the Tilbury Water Treatment Plant) and the solutions including chlorination and the use of intake infiltration sand filters will be discussed.

**ZEBRA MUSSEL VELIGER RESEARCH AT KIRTLAND INTAKE SYSTEM.** Gerald Gubanich, Cleveland Division of Water, Crown Water Treatment Plant, 955 Clague Road, Westlake, OH 44145; Alan Greenberg, J. Giaccia, and W. Mucci, Cleveland Division of Water, Utilities Building, 1201 Lakeside Avenue, Cleveland, OH 44114; Gerald Matisoff, Case Western Reserve University, Department of Geological Sciences, 112 A. W. Smith Building, Cleveland, OH 44106.

A study comparing water from one of Cleveland's pump stations with water in the immediate vicinity of the intake was done in the summer of 1990. The intake was chosen because it was the only one of the city's four intakes with infestation signs.

*Dreissena's* veliger numbers and algal numbers were compared within the pump building to a location just outside the intake's crib. A depth profile was done in the lake using parameters such as dissolved oxygen, temperature, veliger numbers, turbidity, and algal numbers. Because water is entering the crib from two different depths, the profile was used to give an indication from which depth most of the water is entering the crib. The profile was also used to compare the Central Basin of Lake Erie with both the Western and Eastern Basins.

**ZEBRA MUSSEL CONTROL PROGRAM AT CENTERIOR ENERGY CORPORATION.** Joe Szwejkowski and Louise Barton, Centerior Energy, 6200 Oak Tree Boulevard, Room 1-216, Independence, OH 44131.

Zebra mussels present a significant threat to reduce or block flow in power plant systems that use raw water. They were first collected at Centerior at the Perry Nuclear Power Plant (PNPP) in September, 1988. PNPP has developed a three part zebra mussel program that includes monitoring, a chemical treatment program for the 1990 mussel season, and several research projects.

Monitoring methods include the use of artificial substrates, sidestream monitors, plankton nets, and scuba divers in addition to visually inspecting raw water system components when open for maintenance or repair.

PNPP has adopted a proactive approach to mussel control. As a result, a chemical treatment program was established for 1990. It required the use of a proprietary chemical for two treatments during the season, in mid-July and late October.

PNPP has conducted studies on the effectiveness of various applications of chlorine using the plant chlorination and dechlorination systems. Results indicated that although intermittent applications are ineffective for control of adult mussels, continuous chlorination can be effective. An additional study on the effectiveness of intermittent chlorination for control of veligers was conducted during the summer of 1990, as well as a study of the effectiveness of pressure to control zebra mussels.

PNPP is also working in conjunction with the Electric Power Research Institute (EPRI) to evaluate the performance of three proprietary chemicals. The chemicals are being evaluated in sidestream testing apparatus designed to simulate plant conditions. The objectives of the project are focused on optimizing applications toward demonstrating environmental stewardship and cost effectiveness.

Several of Centerior's other facilities have also used a proprietary chemical to treat infested service water systems. These fossil plants are also permitted to use chlorine intermittently as biofouling treatment. The potential use of intermittent chlorination at

these facilities is being investigated as a method of keeping raw water systems free from future infestation, once they have been "cleared" with the proprietary chemical. Although ineffective against adult zebra mussels, intermittent chlorination of service water systems is expected to be lethal to veligers.

**EFFECTS OF LEMMATOXIN (ENDOD) ON ZEBRA MUSSELS.** Harold H. Lee and Aklilu Lemma, University of Toledo, Department of Biology, Toledo, OH 43606.

Berries of the African soap berry, *Phytolacca dodecandra*, commonly known as endod, contain a molluscicidal component, Lemmatoxin, that has a lethal effect on zebra mussels as well as snails. Earlier experiments using static system indicated that 10 to 20 ppm of the solution made from the powder of the dry berries could effectively kill almost 100% of the mussels within 24 hours. Those that were not killed could not attach themselves onto the beakers or to each other. Based on these results, a recycling system was used to test the effectiveness of endod. In one experiment, 50 mussels were placed in the 800 ml cylindrical "pipe" and allow them to acclimatize and to attach for 3 days before treatment with endod. At approximately 50 to 100 ppm, which is high enough to kill all animals in the static system, 36 of the 50 animals were killed within 24 hours and 8 more the next 24 hours. Video tape record shows that the mussels did not close when endod entered the system. Endod powder is now available on demand.

**DIRECT COSTS OF ZEBRA MUSSEL DAMAGE TO FACILITIES AND EQUIPMENT AT LAKE ERIE.** Leroy J. Hushak, The Ohio State University, Ohio Sea Grant College Program, 232 Agricultural Administration, 2120 Fyffe Road, Columbus, OH 43210.

From May to November, several groups who use Lake Erie water were surveyed for zebra mussel damage: commercial shippers, establishments with water intakes (power plants, municipal water systems, industrial users), marinas, charter captains and private boaters. In addition, several port authorities were contacted but indicated no damages or problems.

All 12 of the commercial shippers in the Great Lakes Carriers Association responded. Only \$1,500 in zebra mussel related expenses were reported. Regular maintenance and preventive actions appear adequate for zebra mussel control.

Ohio EPA provided a list of firms with water intakes including municipal water systems, power plants and industrial users in northeast and northwest Ohio. Of nearly 200 establishments, 89 responded that they do not draw water from Lake Erie. Another 52 respondents (35 water systems, 9 power plants, 8 industrial users) do draw water from Lake Erie. Ten stated they had damages and/or cleaning and removal costs due to zebra mussels. Five reported costs ranging from \$250 to \$50,000. Eight took preventive actions in 1989 and 16 as of May, 1990. Extra maintenance

and cleaning were the primary preventive actions. Total expenses reported for zebra mussel damages for 1989 and to May, 1990 was \$210,000, with one firm reporting \$120,000 in research to assess zebra mussel damage and preventive actions.

Of 91 marinas surveyed, 42 responded. Seven reported damages in 1989 and two in 1990. Total reported damages were \$4,600. Six of 42 marina operators in 1989 and 9 in 1990 reported taking preventive actions which cost about \$12,000 in total.

A total of 71 charter captains out of 96 responded to the charter questionnaire. No captains reported zebra mussels causing damage their boats. However, 38 took preventive actions during 1989 and 45 during 1990. Nearly all of these have used preventive paints, and about one-half reported extra maintenance. Mean expenditures for preventive paints were \$121 in 1989 and \$160 in 1990. Extra maintenance expenditures average \$63 in 1989 and \$74 in 1990.

A total of 262 private boaters were surveyed in October, 1990, of which 139 responded. Fifty-one percent used their boat in Lake Erie during 1990 as compared to 52 percent in 1989. One respondent reported zebra mussel damage during 1990, and one during 1989. Fifteen respondents in 1989 and 25 in 1990 took actions to reduce zebra mussel damage to their boats. In 1990, 15 used protective paints, 9 used extra maintenance and cleaning, 6 used on-trailer storage, and 5 used dry rack storage. Mean expenditures for protective paints were \$149 in 1989 and \$111 in 1990. Mean maintenance and cleaning expenses were \$83 in 1989 and \$138 in 1990.

The zebra mussel does not appear to be a major problem for operations of the six groups represented here. Sound maintenance and prevention procedures appear adequate to keep zebra mussel damages under control. However, several charter captains and marina operators reported reduced business because of fear and reduced expenditures by tourists which 13 attributed to the zebra mussel.

**ATTITUDES OF MID-AMERICA BOAT SHOW PATRONS CONCERNING THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*), LAKE ERIE AND GREAT LAKES POLLUTION.** Frank R. Lichtkoppler, The Ohio State University, Ohio Sea Grant College Program, 99 East Erie Street, Painesville, OH 44077; David O. Kelch, The Ohio State University, Ohio Sea Grant College Program, 42110 Russia Road, Elyria, OH 44035.

Because public opinion influences the allocation of resources to manage the Great Lakes it is important to quantify the attitudes of Great Lakes resource users. Six hundred patrons of the 1990 Mid-America Boat Show were surveyed by mail on Great Lakes issues including the invasion of the zebra mussel (*Dreissena polymorpha*), with a response rate of 85 percent. The average respondent was 43.25 years old, male, employed in professional, highly skilled or managerial positions, with household incomes in excess of \$30,000. Almost 78 percent were boat owners. Respondents

agree that pollution by individuals does have an affect on the Great Lakes. They believe that government should be doing more to clean up the Great Lakes and that citizens should be involved in the management of the Great Lakes. The zebra mussel is viewed as a threat to the Lake Erie boating and sportfishing industry. Respondents indicated that they would like to have some of their current tax monies used for research on zebra mussels and that public funding for research on zebra mussels is a wise investment.

**DEVELOPMENT AND USE OF STANDARDIZED ZEBRA MUSSEL MONITORING GUIDELINES.** **J. Ellen Marsden**, Illinois Natural History Survey, Lake Michigan Biological Station, Box 634, Zion, IL 60099.

Information about the spread of zebra mussels is critical for industries and public utilities concerned about water intake systems, and for fisheries agencies concerned about managing the Great Lakes ecosystem. Population density data can be used to track the movement of zebra mussels, to determine what environmental factors influence local population densities, and to determine the efficacy of control measures. Agencies and researchers around the Great Lakes and inland lakes have already initiated a variety of monitoring programs to track the zebra mussel infestation. However, unless a standardized monitoring protocol is used, the information from monitoring stations will be difficult, at best, to interpret. For example, equal numbers of spat settled on PVC versus glass settlement plates may not reflect equal population densities, due to substrate preferences of the spat. Criteria for a standardized sampling protocol include: equipment that is inexpensive, readily obtainable, and simple to build and deploy; techniques that can be used in a variety of field situations; straightforward data collection and analysis methods; and guidelines that can be used by individuals with diverse training backgrounds. Because zebra mussels can be measured at three life stages (as planktonic veligers, during larval settlement onto substrate, or as settled adults), sampling techniques need to be described for each stage. A survey of people involved in zebra mussel monitoring indicated that most people measured veliger densities using 60–64  $\mu\text{m}$  mesh plankton nets, but that sampling of settled spat involved a range of different techniques. Guidelines are being prepared that describe (1) how to sample and count veligers using a plankton net, (2) a sampler for collecting settled spat, using microscope slides as a uniform and readily obtainable substrate and (3) how to count adults settled on natural substrates. The next phase in this project will be the collection of data using the standard techniques in order to refine the techniques and report benchmark measurements of zebra mussel densities.

**WATCHING FOR ZEBRA MUSSELS: WHY CAN'T WE FIND THEM IF THEY'RE ALREADY HERE?** **Clifford Kraft**, Wisconsin Sea Grant Institute, University of Wisconsin, ES-105, Green Bay, WI 54311; **Mary Balcer**, University of Wis-

consin, Center for Lake Superior Studies, Barstow Hall, Superior, WI 54880; **Art Brooks**, University of Wisconsin, Center for Great Lakes Studies, 600 E. Greenfield Avenue, Milwaukee, WI 53204; **Jory Jonas**, University of Wisconsin, Sea Grant Office, ES-105, Green Bay, WI 54311; **Allen H. Miller**, University of Wisconsin, Wisconsin Sea Grant Institute, 1800 University Avenue, Madison, WI 53705; **Hans Pearson**, Silver Lake College, 2406 S. Alverno Road, Manitowoc, WI 54220-9319; **Charles W. Ramcharan**, University of Wisconsin, Department of Zoology, Birge Hall, 430 Lincoln Drive, Madison, WI 53706.

A coordinated sampling program to identify the presence of zebra mussels in Wisconsin waters of Lakes Michigan and Superior was conducted from June through October, 1990. Three sampling methods were used: (1) vertical plankton tows in harbor areas, (2) water intake samples strained through plankton nets and (3) substrate samplers placed in harbor areas. During 1990, the following samples were analyzed as part of the Wisconsin Sea Grant Zebra Mussel Watch: in Wisconsin waters of Lake Michigan—214 harbor substrate samples, 176 harbor plankton tows and 286 water intake samples; in Wisconsin waters of Lake Superior—168 harbor substrate samples, 110 harbor plankton tows and 58 water intake samples. No zebra mussels were found in any of the samples collected from Wisconsin waters of Lake Michigan. Zebra mussels were found in Superior Harbor plankton tows on August 21 at densities of 46–51 per cubic meter, and August 28 at densities of 40 per cubic meter. Recently settled zebra mussels were found on Superior harbor substrates on August 28 (1 mussel, 0.76 mm length), September 6 (3 mussels, 0.44–0.52 mm length), September 24 (5 mussels, 1.08–2.28 mm length) and October 5 (10 mussels, 0.88–2.0 mm length).

Despite the regular observation of adult zebra mussels attached to large boats—as well as observations of small mussel colonies at several locations in the waters of southern Lake Michigan—no zebra mussels were found in any Lake Michigan plankton samples, including those from Green Bay. Our experience suggests that a weekly plankton and substrate sampling program can be used to identify the presence of a reproducing zebra mussel population. Such early identification provides facilities managers information on impending colonization, and provides the basis for initiating remedial actions. However, isolated observations of adult zebra mussels may often provide the first indication of their presence at a particular location.

**CHEMICAL OXIDANTS FOR THE CONTROL OF ZEBRA MUSSEL VELIGERS.** **John E. Van Benschoten**, **James N. Jensen**, and **Thomas J. Brady**, SUNY at Buffalo, Center for Hazardous Waste Mgmt., 207 Jarvis Hall, Buffalo, NY 14260; **Donald P. Lewis** and **John Sferrazza**, Aquatic Sciences Inc., St. Catharines, Ontario.

Since their introduction into Lake St. Clair, zebra mussels (*Dreissena polymorpha*) have spread throughout Lake Erie. The

mussels are now being found in Lakes Ontario and Michigan and it is expected that the mussel will eventually spread to many fresh-water systems in North America. Pipelines appear to offer an idea habitat for *Dreissena* and the prolific nature of the mussel has resulted in restrictions in the hydraulic capacity of some water intakes. For water systems that are not yet heavily infested, methods that prevent veligers from attaching to pipe walls are of interest.

The primary objective of the study was to examine the effectiveness of chemical oxidants for inactivation of zebra mussel larvae. The oxidants studied were chlorine, hydrogen peroxide and ozone.

Experiments were conducted at the Erie County Water Authority's Jerome Van de Water treatment plant located adjacent to the Niagara River in the Town of Tonawanda, New York. The experimental apparatus consisted of a series of baffled, plug flow tanks. Each of the baffled tanks was supplied with a constant flow of Niagara River Water from a constant head tank. One of the baffle tanks served as a control (no oxidant) while three were used in the study, allowing three oxidant dose (three replicates and one control per dose) to be tested simultaneously. Oxidant effectiveness was determined by two methods. One method involved periodic analysis of plexiglass substrates attached to the baffles to assess if the applied oxidant dose was effective in preventing mussel attachment. The second method involved concentration and microscopic examination of suspended veligers to determine oxidant effectiveness. Results obtained using the second method will be presented.

Chlorine was applied continuously for several weeks at nominal free residual concentrations of 0.2, 0.5 and 1.0 mg/L. For each of the doses tested, chlorine was highly effective in killing veligers. Percent kill data showed that a three log reduction (i.e., 99.9 percent kill) was possible with the use of chlorine. Hydrogen peroxide tests were conducted in a pulsed mode where peroxide was added for a 30 minute period every 12 hours. Results indicated that although peroxide was effective in killing veligers, the 2.3 log reduction (95.5% kill) was less than was achieved using the lowest chlorine doses. Ozone experiments were conducted with applied doses of 0.1, 0.2, 0.5 and 1.0 mg/L. Short term tests (3 to 4 hours) were conducted and it was found that removal of veligers from the water column ranged from 50 to 100 percent. Surprisingly, however, examination of veligers in the sediments of the tanks revealed 30 to 50 percent survival rate. The implications of this finding in terms of practical application of ozone will be discussed.

**THE RANGE EXTENSION OF THE ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) INTO THE INLAND WATERS OF NEW YORK STATE.** Cameron L. Lange and Roberta K. Cap, Acres International Corporation, 140 John James Audubon Parkway, Amherst, NY 14228-1180.

The possibility of a range extension of the zebra mussel (*Dreissena polymorpha*) from the Great Lakes drainage into adjacent drainages is a major concern of raw water users. Likely mechanisms for transport are by active transfer through human activities or transfer through interconnecting canals. In New York State, the State Barge Canal was considered the most likely route for a zebra mussel range extension, since in the spring the Canal is filled with water from the Niagara River, where the mussels have become well established. In April 1990 a monthly sampling survey was initiated to determine the extent of the zebra mussel's spread into the New York State inland waters. Nine sampling stations were set along the Barge Canal. Four stations were located in the Cayuga-Seneca Canal/Finger Lakes System and one on the Oswego Canal. A total of eight other stations were sampled on the Susquehanna, Hudson River and Delaware River drainage systems. In July, veligers and recently settled juveniles were collected at Lockport and Albion on the State Barge Canal and on the Genesee River in Rochester, New York, about one mile downstream from the confluence with the Canal. The Rochester station is about 80 canal miles from the Niagara River. Veliger numbers peak at 11,250/m<sup>2</sup> in the August sample collected in Lockport. Settlement peaked at 64,900 juveniles/m<sup>2</sup> on a substrate collected at Albion in August. Through November 1990, zebra mussels were not found at any sampling station east of Rochester.

**POTAMOCORBULA AMURENSIS, A RECENTLY INTRODUCED ASIAN CLAM, HAS HAD DRAMATIC EFFECTS ON THE PHYTOPLANKTON BIOMASS AND PRODUCTION IN NORTHERN SAN FRANCISCO BAY.** James Cloern and Andrea Alpine, U.S. Geological Survey, MS 496, 345 Middlefield Road, Menlo Park, CA 94025.

*Potamocorbula amurensis*, a benthic suspension feeding bivalve, accidentally introduced in San Francisco Bay in 1986, spread rapidly throughout the estuary with dramatic ecological consequences. Field and laboratory evidence suggest that this species is capable of consuming most of the phytoplankton produced in northern San Francisco Bay. Prior to its introduction, the long-term record of chlorophyll *a* showed a repeatable pattern of summer maximum in phytoplankton biomass (30 to 40 mg/m<sup>3</sup> in Suisun Bay for two to three months). Beginning in 1987, *P. amurensis* had become well established, the summer maximum in biomass never appeared and chlorophyll *a* levels have remained at their winter levels of less than 3 mg/m<sup>3</sup> throughout 1988, 1989 and 1990.

To further define the effects of this invasion we initiated a study in 1988 to examine rates of primary productivity and related parameters to compare with pre-invasion levels. Daily rates of primary productivity were much lower in 1988 compared to rates measured in 1980. These lowered daily productivities led to a dramatic decline in annual production—1988 production was only

20 g C/m<sup>2</sup> compared with average annual production of 110 g C/m<sup>2</sup> during pre-invasion years.

We believe this decline in production is a consequence of the consumption of phytoplankton by *P. amurensis*. Other factors capable of reducing productivity are not likely. Nutrients are abundant at all times and could not be considered limiting. Maximum rates of primary productivity per unit biomass were no different from pre-invasion years, and as in pre-invasion years productivity was highly correlated with biomass and available light. Preliminary measurements of *P. amurensis* feeding rates and densities in the field are sufficient to account for the reduction in phytoplankton biomass in northern San Francisco Bay during the past four years.

**AN ASIAN BIVALVE, *POTAMOCORBULA AMURENSIS*, INVADES SAN FRANCISCO BAY WITH REMARKABLE SPEED AND SUCCESS.** Janet K. Thompson and L. E. Schemel, U.S. Geological Survey, MS 496, 345 Middlefield Road, Menlo Park, CA 94036; Susan Jerrine Nichols, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI 48105.

San Francisco Bay has a long history of species introductions. However, the recently introduced Asian bivalve, *Potamocorbula amurensis*, may be unlike previously introduced species in that it has rapidly spread throughout the bay, irrespective of sediment type, water depth, and salinity. In addition it seems to be altering existing benthic and pelagic communities in ways not previously seen in the bay. We have established the time (fall 1986) and place of introduction (in northern bay) and have tracked its spread within the bay. Within one year of its first sighting, the clam was found in high abundances (>25,000 m<sup>2</sup>) at many sites and is now found throughout the bay. Using long-term data collected near the location where *P. amurensis* was first seen, we have documented a major and rapid shift in benthic community structure. The previous community was one with variable species composition dependent on seasonal and inter-annual patterns of river inflow (salinity). Within one year of the clam's first sighting, this community was nearly replaced by *P. amurensis* (now contributing >95% of total individuals and biomass). *P. amurensis* has also become established at many locations in the southern bay, an area where the benthic communities are more temporally stable than the pre-*P. amurensis* benthic communities in the northern bay.

*P. amurensis* is a suspension feeder that, in its present abundances may consume a major fraction of the phytoplankton produced in this shallow estuary and, thereby, change the trophic dynamics of part or all of the estuary. The successful encroachment of this species into the existing benthic community, to the point of displacing established species, may be cause for concern if it spreads to nearby estuaries that support commercial shellfisheries.

**DREISSENA POLYMORPHA COLONIES ENCRUSTING NATIVE UNIONID BIVALVES PRODUCE SPECIES-SPECIFIC AND SEX-SPECIFIC EFFECTS.** Wendell R. Haag, David J. Berg, and David W. Garton, The Ohio State University, Department of Zoology, 1735 Neil Avenue, Columbus, OH 43210.

*Dreissena polymorpha* from large colonies on unionid bivalves in areas where they are sympatric. The recent appearance of *Dreissena* in North America has generated concern regarding the possible effects of *Dreissena* on populations of native bivalves already stressed by environmental degradation. We used experimental and survey approaches to evaluate the effects of *Dreissena* colonies on unionid species. In a field experiment, *Amblema plicata* showed no difference in survival between *Dreissena* encrusted and unencrusted (control) treatments. In contrast, 83.3% of encrusted female *Lampsilis radiata* died as opposed to 31.6% mortality in male and female control animals ( $p < 0.0002$ ). Mortality in encrusted males was not different from control animals ( $p = 0.37$ ), suggesting that stresses related to brooding and release of glochidia (occurring during the experiment) were compounded by stresses incurred from *Dreissena* encrustation, producing lethal effects.

A survey of three sites in the western basin of Lake Erie showed that mortality was unevenly distributed among encrusted animals of different species. *Anodonta grandis* and *Leptodea fragilis* were present in higher proportions among recently dead unionids than among living animals. Conversely, *A. plicata* and *Quadrula quadrula* were present in lower proportions in dead animals than live. Proportions of living and dead *Potamillus alatus* and *L. radiata* were not significantly different at two sites.

We propose two possible mechanisms by which *Dreissena* may affect survival of unionids: 1) colonies add substantial weight to the unionid, increasing the metabolic cost of maintaining feeding position in the substrate, or 2) the filtering activities of the large number of *Dreissena* on a unionid interfere with feeding by the unionid. Our initial results favor the former hypothesis because mortality is high in thin-shelled unionids (*Anodonta*, *Leptodea*), less severe in species of intermediate shell weight (*Lampsilis*, *Potamillus*) and low in heavy-shelled species (*Amblema*, *Quadrula*). We are further testing these hypotheses by assessing glycogen and lipid reserves as a measure of nutritive state in experimentally and naturally encrusted and unencrusted *A. plicata* and *L. radiata* and are conducting an experimental analysis of the effects of increased weight on unionid mortality.

This project was supported by Ohio Sea Grant.

**ADHESION STRENGTH IN ZEBRA MUSSELS: PRELIMINARY DATA USING A ROTATING DISK APPARATUS.** Joseph Daniel Ackerman, C. R. Ethier, D. G. Allen, and J. K. Spelt, University of Toronto, Mechanical Engineering, 5 King's College Road, Toronto, Ontario M5S 1A4.

The ability of zebra mussels (*Dreissena polymorpha*) to adhere to hard surfaces is an important factor in the explosive population growth of this species. The colonization of natural substrates such as rocks, macrophytes, and the exoskeletons of "large" invertebrates has created ecological problems. Moreover, there has been a large economic impact associated with the colonization of artificial substrates such as boats, pilings, and municipal and industrial water intake systems.

We have examined the strength of adhesion in zebra mussels and related this parameter to residence time of the mussel and to the surface properties of the attachment substrate. This adhesion strength cannot be easily tested using conventional mechanical equipment, and we have, therefore, developed a rotating disk test system. The adhesion strength is inferred from the fluid force required to detach (or remove) mussels, which have been grown on disks of different materials. This method is effective, since the fluid force varies with radial position on the disk, so that a single experiment provides a range of fluid shearing forces.

We have successfully used the rotating disk to detach small mussels (post-veligers; 500–1000  $\mu\text{m}$  long) from plexiglass, stainless steel, PVC and aluminum disks 20 cm in diameter. Detachment of post veligers at large radii (e.g. 70 mm) occurred at rotation rates of 300 Rpm, corresponding to a shearing stress of 10 Pa. Shear stresses on the disk were calculated at different radial locations and rotation rates using well known fluid mechanical models. As expected, greater rotation rates were needed to detach mussels that were located closer to the center of the disks. Preliminary results indicate a great deal of variation in the strength of adhesion; however, we note that the upper limit of 150 Pa was sufficient to remove most mussels. This preliminary result could serve as a minimum detachment criterion for mitigation guidelines.

**BIOLOGICAL CONTROL OF ZEBRA MUSSELS: USE OF PARASITES AND TOXIC MICROORGANISMS.** Daniel P. Molloy, NYS Museum—Biological Survey, Field Research Laboratory, RD 1, Box 151, Cambridge, NY 12816.

A variety of control measures will have to be developed to manage zebra mussels, and biological control—the use of other organisms to reduce population densities of this mussel—cannot be overlooked. The New York State Museum, following its development of a biological method of black fly control in New York State, is initiating two research projects against this new invertebrate pest.

One approach to developing a biological control method for zebra mussels is the search for parasites which naturally kill them. Zebra mussel populations in the Great Lakes may be harboring parasites, and these and other North American mussel populations will be examined. Parasites of any animal group, however, are most likely to be found where the animal group has lived the longest. Eastern Europe and Western Asian countries, therefore, are the location most likely to contain zebra mussels dying of parasitic diseases since they have been in these regions for centuries. To limit the parasite search to the Great Lakes and to exclude these foreign regions would be a mistake. While some parasites from these foreign regions have been reported, their zebra mussel populations have not been comprehensively surveyed to date. These foreign parasites need to be taxonomically identified, life cycles elucidated, and their lack of infectivity to North America nontarget fauna determined in rigorous laboratory tests. Once the low nontarget risk is established, these European/Asian parasites of zebra mussels should be transferred into the Great Lakes. The successful establishment of such parasites would act as a buffer to the rapid population growth of zebra mussel populations in North America.

Another approach is the laboratory screening of microorganisms to find one that is lethal upon ingestion by zebra mussels, but which is nontoxic to other Great Lakes fauna. These candidate control microorganisms would not be "natural" parasites of zebra mussels, but rather naturally occurring soil and water microbes, which just by "chance" happen to be highly toxic to zebra mussels when the mussels are exposed to artificially high densities of the microbe. Sound like impossible odds? Not really. This is exactly the research approach that identified a soil microbe, *Bacillus thuringiensis* var *israelensis* (BTI) as the safest and most effective commercial method for the control of another aquatic invertebrate—the black fly. BTI is now being used worldwide for the control of these pest flies. Both zebra mussels and larval black flies are similar in that they are filter-feeding invertebrates which prefer lotic habitats. The microbe BTI, although a highly poisonous bacterium to larval black flies, does not normally kill them, since it lives in the soil in very low densities. When applied in large numbers to waters where black fly larvae live, however, they inadvertently ingest BTI and die within hours, virtually leaving all other organisms undisturbed. BTI would be useless against zebra mussels, but another microorganism which at artificially high densities is poisonous to zebra mussels undoubtedly exists in nature; the task now is to begin the search for it.

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## SCALLOPS

**FOOD QUALITY, FEEDING ACTIVITY AND ENERGY BALANCE IN THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS*.** Gregory S. Bacon\* and Bruce A. MacDonald, Marine Sciences Research Laboratory, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, N.F., Canada A1C 5S7.

The giant scallop *Placopecten magellanicus*, a benthic suspension-feeder, is exposed to a food supply that fluctuates unpredictably in both quantity and quality. Many species of bivalves are capable of maintaining the flow of energy into growth and reproduction by adjusting the efficiency of feeding or metabolic activity but little is known about the ability of *P. magellanicus* to exploit its food resource. Mixtures of microalgae and silica were delivered to scallops held in flow through chambers in the laboratory at concentrations ranging from 1 to 12 mg l<sup>-1</sup> and organic fractions ranging from 20 to 90%. The quantity and composition of diets was similar to particle concentrations and ratios of organic to inorganic components actually measured in the natural environment. Scope for growth was estimated for juvenile scallops (40–50 mm shell height) by determining energy gain through feeding and losses associated with respiration and ammonia excretion under these experimental food conditions. These results will be discussed in context of feeding strategies for this species.

### TROPHIC SOURCES AND TRANSFER MECHANISMS TO THE DEVELOPING GAMETES OF *PECTEN MAXIMUS*.

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Ultrastructural, histological and histochemical studies were performed on the gonad of adult *Pecten maximus* at various intervals during the reproductive cycle in St. Brieuc Bay, France, in order to better understand the sources and transfers of energy to developing gametes in scallops. In addition to the well-known pathways of energy acquisition through feeding and transfer of somatic reserves, a number of novel pathways were demonstrated. These were grouped into two categories: atretic recycling and intestinal loop transfer. Evidence is presented for (1) the recovery of lytic material (resulting from gamete atresia) in the gonad acini, gonoducts, and integument; and (2) the direct transfer of metabolites from the gonad intestinal loop to the developing gametes via vesicular cell-haemocyte couples, which appear to follow fibrous pathways within the loose connective tissue extending from the base of the intestinal epithelium to the acini. A summary of the sources and transfer mechanisms of the energy exchanges involving the developing gametes is presented.

### PREDATORY RISK OF JUVENILE BAY SCALLOPS, *ARGOPECTEN IRRADIANS*, IN EELGRASS HABITAT.

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Above-bottom attachment on eelgrass blades offers post-settlement scallops partial refuge from benthic predators. To assess predatory risk in relation to scallop size, temporal patterns in attachment position on eelgrass were determined for natural postset in two Long Island bays differing markedly in eelgrass density. Once scallops attained 12 mm, the proportion of individuals attached above-bottom decreased rapidly with increasing size over a 5 wk period. By early October nearly 100% of the population (mean size = 30 mm) had moved to the bottom. The rate and timing of relocation to the bottom was remarkably consistent between the two embayments. Whereas percent attachment was highly size-dependent, there was no correlation between height attained above-bottom and scallop size.

Size-specific losses of scallops due to emigration and/or predation were determined by both tethering and free release of two size classes (12–14 and 20–22 mm) within contrasting eelgrass habitat in Hallock Bay, L.I., where mud crabs, *Dyspanopeus sayi*, were identified as the most abundant predator. There was a strong seasonal effect on predation of the larger scallops: higher mortalities in late summer-early fall are attributed to the appearance of blue crabs, and/or increasing vulnerability to mud crabs, which grew at a rate of 3 mm in carapace width per month. Recovery of free planted scallops was significantly greater for 20 than 14 mm size classes, but both suffered heavy losses in early fall (85 and 99% respectively over a 2 wk period). Field observations are consistent with laboratory results indicating that only scallops larger than 26 mm achieve complete size refuge from *D. sayi*. The significance of these studies in assessing seasonal predatory risk of natural scallop populations, as well as the probability of success of reseeded efforts is discussed.

### CONSIDERING A SEMI-ANNUAL REPRODUCTIVE CYCLE FOR THE SEA SCALLOP (*PLACOPECTEN MAGELLANICUS*) ON GEORGES BANK.

Claudio Dibacco, Department of Oceanography, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

Considerable effort goes into managing the world's largest natural sea scallop (*Placopecten magellanicus*) resource located on Georges Bank. A prerequisite in attaining and maintaining a stable fishery is an accurate age assessment of stocks, yet standard aging methodology remains a point contention. The controversy lies in the number and length of spawning periods characterizing sea scallop reproduction in the course of a year. Initial studies report a short synchronized fall spawning season for Georges Bank scallops. Subsequent considerations suggest more protracted spawning events with respect to changing environmental condi-

tions, as well as the possibility of spring spawning in the areas of Newfoundland, Maine and the mid-Atlantic region.

Gonado-somatic indices, determined for Georges Bank scallops over the past four years, have revealed a bimodal reproductive strategy. Preliminary analysis of histological data collected in late spring and early summer (1990) revealed a high percentage of individuals possessing developed gametes, further supporting the possibility that sea scallops release viable gametes at this time. Recognition of a semiannual reproductive strategy would influence not only biological considerations, but also the management and regulation of the commercial scallop fishery.

**INTERACTIONS BETWEEN AT-SEA HANDLING PRACTICES, REGULATORY CONSTRAINTS AND SEA SCALLOP, *P. MAGELLANICUS* (GMELIN), MEAT QUALITY AND VOLUME.** William D. Dupaul,\* James E. Kirkley, and Robert A. Fisher, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Weight changes in shucked sea scallops during vessel stowage was determined for three trips during the summer months of 1989 and 1990. Bags (N = 576) of scallop meats (36–40 lbs.) were weighed prior to stowage in ice holds and then reweighed at off-loading. The holding (deck treatments) of shucked scallops on deck from 1–8 hours prior to bagging resulted in weight gains of 7.0–22% and had significant impact on the weight gain (or loss) of scallop meats during stowage. Deck treatments or holding conditions tested included ice:seawater (1:2), sea water, ice:freshwater and dry. Temperatures were determined for on-deck conditions and for bag cooling rates during stowage. On deck thermal abuse (greater than 72°F) of scallop meats resulted in diminished weight uptake during stowage and adverse product integrity. Weight gains during deck treatments and stowage can allow a modification of industry practices relative to the minimum size scallop shucked on the harvesting vessel. The restrictive nature of meat count regulations coupled with the current condition of the sea scallop resource, necessitated that commercial practices worked counter to desired regulatory control and preferred product quality.

**TEMPORAL VARIATIONS IN SPAWNING BEHAVIOR OF SEA SCALLOPS, *PLACOPECTEN MAGELLANICUS* (GMELIN), IN THE MID-ATLANTIC RESOURCE AREA.** James E. Kirkley\* and William D. Dupaul, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Sea scallops, *Placopecten magellanicus*, from the mid-Atlantic resource area appear to spawn at least twice a year. There is, however, considerable variation in spawning behavior between years. Knowledge of this variation may be quite important to management agencies concerned with maximizing yield-per-recruit via restrictions on meat counts or temporal restrictions on catch

and effort. In this paper, the temporal variations are identified by conventional time-series analysis of monthly gonadal indices for 1987–1990. Seasonal and cyclical behavior are identified. It is concluded that management agencies concerned with maximizing yield-per-recruit need to closely monitor spawning behavior.

**SHORT- AND LONG-TERM TEMPORAL PATTERNS IN THE REPRODUCTIVE CYCLE OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*, FROM PASSAMAQUODDY BAY, N.B., CANADA.** G. Jay Parsons,\* Dept. of Zoology, University of Guelph, Guelph, Ont., N1G 2W1; Shawn M. C. Robinson, Ross A. Chandler, Dept. of Fisheries and Oceans, St. Andrews, N.B., E0G 2X0; Leslie A. Davison, Mark Lanteigne, Dept. of Fisheries and Oceans, P.O. Box 5030, Moncton, N.B. E1C 9B6; and Michael J. Dadswell, Dept. of Biology, Acadia University, Wolfville, N.S. B0P 1X0.

The reproductive cycle of the sea scallop, *Placopecten magellanicus* was examined over a 13 year period from 1978–1990 at several sites within Passamaquoddy Bay, New Brunswick. Gonad weight and gono-somatic index (GSI) were obtained from scallop samples collected on approximately a monthly basis. From 1985–1990 samples were collected weekly during the gonad ripening and spawning period. The annual reproductive cycle in Passamaquoddy Bay had one main spawning period from July to September with peak spawning occurring during August. Spawning lasted from 20 to 35 days. Within this period, spawning was not a continuous process, but rather was intermittent, and was manifested as 2–3 spawning events. Annual differences in GSI maxima ranged from 16.6% in 1980 to 28.5% in 1985. The maximum reproductive level (highest observed GSI) for this population was 43%. Onset, duration, and periodicity of spawning were consistent between years and between sites within Passamaquoddy Bay. Juvenile scallops initiated gonad development at 40 mm (shell height) and the majority were fully mature by 60 mm. This corresponded to an age of less than one to two years. Annually, male adult scallops ripened at a faster rate and attained a higher GSI than females. Gonad ripening and spawning were synchronous between sexes.

**SPATIAL PATTERNS OF SPAT SETTLEMENT IN THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*, COMPARED TO HYDROGRAPHIC CONDITIONS IN PASSAMAQUODDY BAY, NEW BRUNSWICK, CANADA.** M. C. Shawn Robinson,\* James D. Martin, and Ross A. Chandler, Dept. Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick, E0G 2X0, Canada. G. Jay Parsons, Dept. Zoology, Univ. Guelph, Ont. N1G 2W1.

In 1989 and 1990, a survey was conducted to examine patterns of spat settlement in the sea scallop, *Placopecten magellanicus*. Standard Japanese scallop spat collection bags were deployed in a uniform grid pattern around Passamaquoddy Bay approximately 2 m above the bottom just prior to settlement of the spat in September. Temperature and salinity profiles were taken at each of

the spat collection sites on a monthly basis to document temperature regimes and the extent of mixing of the water column. Results indicated that settlement patterns were nonuniform and that the highest settlement densities were found in the upper part of Passamaquoddy Bay. Areas of highest settlement had densities of spat up to 3000 per bag, had the largest individuals, and were in the areas with the highest water temperatures. There was a 2 to 3 fold increase in the numbers of scallop spat collected in 1990 compared to 1989. Observed patterns of spat distribution corresponded very well to measured hydrographic properties and appeared to relate to the circulation of a known gyre and prevailing wind patterns at the time of settlement.

#### PARTICLE SELECTION BY THREE SPECIES OF SCALLOPS.

**Sandra E. Shumway**,\* Department of Marine Resources, West Boothbay Harbor, Maine, 04575; **T. L. Cucci**, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, 04575; **M. P. Lesser**, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, 04575; **N. Bourne**, Pacific Biological Station, Nanaimo, British Columbia, V9R 5K6, Canada; **B. Bunting**, Island Scallops Ltd., West Island Highway, RR 3, Site 327, Qualicum Beach, B.C., Canada V0R 2T0.

Juvenile scallops (<2 mm shell height) of three species (*Placopecten magellanicus*, *Patinopecten yessoensis*, *Argopecten irradians*) were fed mixed, unialgal cultures. A total of six algal clones were fed simultaneously and clearance rates monitored using flow cytometric techniques. In addition, scallops were presented with natural assemblages of particulate matter as a food source. Data are presented on differences in clearance rates for the individual algal species as well as size-related differences and uptake of chlorophyll vs. non-chlorophyll cells both within and between scallop species. Significant differences in clearance rates of individual algal species have been found within and between scallop species. Particle selection does not appear to be based upon size alone and is apparently based on characteristics of the algae other than size, or by pre-ingestive sorting by juvenile scallops.

#### RESEEDING EFFORTS AND THE STATUS OF BAY SCALLOP POPULATIONS IN NEW YORK FOLLOWING THE APPEARANCE OF BROWN TIDE.

**Stephen T. Tettelbach**,\* Long Island University, Southampton, NY 11968; **Peter Wenzel**, Long Island Green Seal Committee, Southold, NY 11971.

Populations of the bay scallop (*Argopecten irradians irradians*) in Long Island, New York were decimated after extensive blooms of *Aureococcus anophagefferens* ("brown tide") occurred between 1985–88. Scallop mortality in 1985 was most severe in western Peconic Bays, but populations in eastern Peconic Bays declined dramatically following poor recruitment between

1985–87. By the winter of 1987–88, virtually no native scallop stock remained in the Peconic Bay system. A small, natural population centered in Napeague Harbor, an area less impacted by *Aureococcus* blooms, persisted through this period.

Extensive reseeding of hatchery-reared scallops in the Peconic Bays was initiated by the Long Island Green Seal Committee in 1986. Twenty-mm seed free-planted in late October/early November 1986 survived at one of three sites to spawn the following July. *Aureococcus* bloom conditions which coincided with this spawning apparently thwarted recruitment. Twenty-mm seed planted in mid-September 1987 were wiped out within one month; shell fragments implicated crabs as the primary cause of mortality. In mid-October 1988, 30-mm scallops were seeded at six sites. Mean survival until the following summer ranged from 0–12%. Spawn of these surviving scallops is thought to have contributed significantly to the scallop set which occurred throughout the eastern Peconic Bays in 1989. Observations of a small scallop set in the western Peconic Bays and very heavy recruitment farther east suggest that Long Island bay scallop populations are recovering.

#### DOES THE BEHAVIOR OF SEA SCALLOP LARVAE INFLUENCE THEIR DISPERSAL?

**M. John Tremblay**, Halifax Fisheries Research Laboratory, Biological Sciences Branch, Dept. of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7.

Sea scallop (*Placopecten magellanicus*) larvae undertake a small amplitude (ca 5 m) diel vertical migration in some parts of their range, but such a migration was not detected on Georges Bank, perhaps because of insufficient sampling resolution. In the shallower, well mixed parts of Georges Bank, sea scallop larvae are distributed evenly with depth, and estimates of vertical mixing rates are greater than the estimates of larval swimming speed. In those areas of Georges Bank with some density stratification, larvae are often aggregated in the region of the pycnocline. Estimated vertical mixing rates for these weakly stratified areas are closer to the swimming speed of sea scallop larvae. The aggregation of sea scallop larvae near the pycnocline does not coincide with increased biomass of potential food (small phytoplankton measured by chl *a* < 15  $\mu$ m), and the aggregation may be the result of lower turbulence in this part of the water column.

The mean centre of mass of sea scallop larvae in the weakly stratified parts of Georges Bank was 24.3 m (N = 25, SD = 7.2). Current speeds at this depth are lower than in the upper 5–10 m, and therefore transport via currents should be less than if larvae were aggregated in the surface layer. From this perspective then, the behavior of sea scallop larvae should reduce the rate of dispersal on Georges Bank. However sea scallop larvae do not appear to 'use' the circulation through complex vertical migrations, and the role of behavior in larval dispersal is thus less than it could be. Given that sea scallop larvae appear to be retained on Georges Bank without complex swimming behavior, the physical

retentiveness of Georges Bank may be the dominant feature determining the retention of stronger swimming zooplankters as well.

**SELF-FERTILIZATION IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*.** Ami E. Wilbur\* and Patrick M. Gaffney, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Self-fertilization in hermaphroditic bivalves is generally thought to only occur in small, brooding species. Other hermaphroditic species, which spawn eggs to be fertilized externally are only thought to self-fertilize inadvertently, and selfed progeny are presumed to be less fit. Preliminary work with the bay scallop, *Argopecten irradians*, suggests that this may not be the case for this species. A significant portion of the eggs collected immediately upon release from isolated spawning scallops were fertilized. Selfed larval cultures were found to grow as well as outcrossed larval cultures. This result, however, may be confounded by an undetermined proportion of selfed larvae in outcrossed families. Electrophoretic types at 7 months of age has revealed a significant portion of an outcrossed family to be the products of self-fertilization. Comparison of selfed and outcrossed progeny for shell dimensions (length, height and width) suggests a lack of significant inbreeding depression.

## GENERAL BIOLOGY OF BIVALVES

**BIOTIC AND ABIOTIC FACTORS INFLUENCING THE BURROWING OF THE SOFTSHELL CLAM, *MYA ARENARIA*.** Cal Baier-Anderson, University of Maine, Orono, ME 04469.

Clamflat seeding, using hatchery-reared softshell clams (*Mya arenaria*) has the potential to increase clamflat productivity in over-harvested areas. Seed clams must be capable of rapid burrowing into the sediment to reduce losses to predation. Two laboratory experiments, designed to characterize the burrowing ability of softshell clams, were performed in 1989 at the Beal's Island Regional Shellfish Hatchery. In the first experiment, burrowing times of hatchery-reared clams ( $\bar{x}$  SL = 11.36 mm, SE = 0.21, N = 96) were compared to clams from a local clamflat (e.g. "natural" clams,  $\bar{x}$  SL = 12.11 mm, SE = 0.34, N = 38). The second experiment compared burrowing times of hatchery clams ( $\bar{x}$  SL = 20.08 mm, SE = 0.10, N = 144) and natural clams ( $\bar{x}$  SL = 21.40 mm, SE = 0.19, N = 71) in three substrates: clay, sand and silt.

All burrowing time data was analyzed using survivor analysis (SAS Lifetest). In the first test, hatchery-reared clams burrowed significantly faster than natural clams. In the second experiment, general trends indicate that 1) hatchery clams burrow significantly faster than natural clams, and 2) clams burrow faster in sand than

in silt or clay, and faster in silt than in clay. These results will be discussed in terms of clam age, length and implications for clamflat seeding.

**A LIGHT AND SCANNING ELECTRON MICROSCOPE STUDY OF THE KIDNEY OF THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*.** A. F. Eble\* and C. Ellison, Dept. Biology, Trenton State College, Trenton, NJ 08650-4700.

The shell-side epithelium is contiguous with the mantle and has rugae-like folds over the lower half of its surface. Deeper portions of the folds are lined with cilia; crests lack cilia. This epithelium is a simple, tall columnar containing vacuolated cells. The gill-side epithelium faces the mantle cavity and helps to anchor the anterior extremity of the gills; the epithelium is a simple, low columnar covered with large cilia.

Renal tubules are arranged as a compound gland. Concretions of various sizes can be seen occupying apical portions of tubule cells. Concretions and cellular debris are frequently seen in tubule lumina suggesting apocrine-type secretion by tubule cells. Main ducts leading from kidney have been investigated using a variety of histochemical procedures.

**LECTIN CONTENT AND SPECIFICITY IN SERUM AND MUCUS OF OYSTERS.** William S. Fisher,\* U.S. Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, FL 32561.

Lectins are protein complexes with carbohydrate-specific binding properties that may function in marine invertebrates as nonself recognition molecules and opsonins. Lectin activity has been demonstrated in sera and/or homocytes of many marine invertebrates including oysters *Crassostrea virginica*. Lectins can be detected by agglutination of homologous particles; agglutination is dependent on the specific interaction of a lectin with surface carbohydrates. Serum from *C. virginica* has been shown to agglutinate a broad range of vertebrate erythrocytes and bacteria while retaining a high degree of specificity. For example, one oyster lectin strongly agglutinated *Vibrio cholerae* O1 but did not recognize the non-O1 serotype. It is noteworthy that other *Vibrio* species known to cause disease in humans and marine organisms (*V. alginolyticus*, *V. damsela*, *V. parahemolyticus* and *V. vulnificus*) were not agglutinated by oyster serum.

Mucus on the organ surfaces of oysters exhibited agglutinating activity that was similar in specificity to serum from the same animal. Lectins appeared to be associated with homogenates of mantle, gill and digestive gland, but not with adductor muscle. Lectin presence in the mucus may function in external defense or food selection. It has been generally accepted that leucocytes produce serum lectins, yet this recent evidence implies that lectins occur both externally and internally and may be secreted by several tissues.

**A NEW METHOD FOR DIRECTLY MEASURING BIVALVE PUMPING VELOCITY.** Elizabeth J. Turner, Institute of Marine & Coastal Sciences, Rutgers University, New Brunswick, NJ 08903.

A drawback to studies of bivalve filtration has been the lack of a suitable method for directly measuring pumping velocity. Estimates of filtration rates based on clearance of particles from suspension, or O<sub>2</sub> consumption, cannot be used in field situations or in laboratory flows typical of a benthic environment. I present a method of directly monitoring the velocity of water flowing through a clam's valves, based upon a heated-bead thermistor flowmeter. A heated-bead thermistor, connected to a nylon bolt, can be inserted into the mantle cavity of a clam by drilling the shell, puncturing the mantle and inserting the bead through the hole. A nylon nut, cemented around the hole in the shell, keeps the thermistor in place. Preliminary data from *Mercenaria mercenaria* show no differences in average pumping velocity between external flows of 20 cm/s and 30 cm/s. Pseudofeces production is easily monitored with the flowmeter, and is extremely regular in still water and in both 20 cm/s and 30 cm/s flows. However, the time elapsed between pseudofeces ejections is longer in the faster flow. The development of this monitoring method allows separation of the effects of flow from the effects of suspended particulates on bivalve filtration rates.

**ANALYSIS OF THE MICROSTRUCTURE AND FRAGILITY OF THE SHELLS OF PACIFIC GEODUCK (*PANOPEA ABRUPTA*) REARED IN ARTIFICIAL NURSERIES.** Donald E. Velasquez,\* School of Fisheries, University of Washington, Seattle WA 98195.

The state of Washington is currently involved in a program aimed at artificial enhancement of harvestable stocks of geoduck clams. With this goal in mind, three main problems have surfaced: consistent production of sufficient plantable seed clams, shell fragility in these clams, and very heavy predation mortality for the first year after planting. Two nurseries were utilized and evaluated during the spring and summer of 1990 in the Puget Sound area.

This research is primarily directed toward the problem of shell fragility which has been apparent in some nursery-produced seed. An experiment under artificial conditions is planned to assess if shell breakage contributes to the subsequent problem of predation mortality. Shell quality of seed clams taken from both nurseries will be correlated to information obtained from scanning electron micrographs of valve sections.

A secondary goal of this research is to provide basic information on geoduck shell structure. While current theory suggests that periods of anaerobic respiration are recorded in microgrowth increments of the bivalve shell, it is unclear to what extent these are recorded in gaper clams. Natural subtidal and low intertidal groups of geoduck are analyzed and compared via acetate peel technique to determine whether exposure episodes are evident in the shell record.

## BIVALVE EARLY LIFE HISTORY

**ALTERNATIVES TO CLAMSHELL FOR OYSTER SPAT PRODUCTION.** Robert C. Broadhurst III, Thomas M. Soniat,\* and Edward L. Haywood III, Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148.

The effectiveness of clamshell, limestone, gravel, and concrete as substrate for settlement of the Eastern oyster, *Crassostrea virginica* (Gmelin, 1791), was compared in field and laboratory experiments. A method to produce cultchless oysters using gypsum was also investigated. Setting rates observed on limestone in the field experiment were approximately 1.9 times those on clamshell, and 8.2 those on gravel. In the laboratory experiment, limestone caught approximately 1.9 times as many spat as concrete, and 7.4 times more than gravel. Setting rates on gypsum matched those on clamshell in hatchery experiments. (All statistical tests employed non-parametric, one-way analysis of variance;  $p < 0.05$ ). Larvae metamorphosed and subsequently detached from dissolving gypsum, thus providing cultchless spat.

**EVALUATION OF NILE RED AS A FLUORESCENT, LIPID SPECIFIC STAIN FOR USE WITH INTACT BIVALVE LARVAE.** Laura L. Castell and Roger Mann,\* Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Previous studies have demonstrated the value of lipid content of bivalve larvae as an indicator of physiological condition. The visual, lipid specific stain Oil Red O can be used as a convenient qualitative indicator without complex instrumentation. Accurate quantification can be effected using microphotometry but is tedious and time consuming, requiring six photometric readings at three wavelengths per larvae. We present an evaluation of Nile Red, a fluorescent lipid specific stain, as an alternative to Oil Red O. Accurate quantification is available through microfluorometry using only one reading per larvae; however, the method is very sensitive to variation in larval fixation and staining protocols. Long term storage of larvae prior to analysis does not appear feasible, although some level of automation may facilitate rapid sample analysis.

**EELGRASS AS A LARVAL TRAP: THE "HONAMI" EFFECT.** Heidi Hoven,\* Dep't. of Plant Biology and Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH 03824; Ray Grizzle, Livingston University, Livingston, AL 35470; Frederick Short, Dep't. of Natural Resources and Jackson Estuarine Laboratory/Center for Marine Biology, University of New Hampshire; Linda Kindblom, University of Maine, Orono, ME 04469.

Our observations of the *Zostera marina* (eelgrass) canopy undulating in response to rapid tidal currents ("honami"), and the settlement patterns of *Mytilus edulis* (blue mussel) onto these

blades of eelgrass suggest a potentially very important hydrodynamical effect on larval settlement. Early post-larval and juvenile mussels consistently occurred in substantially higher abundances at the distal tips (top 30 cm) of eelgrass compared to the lower portions of the blades. Larval settlement of this type is found extensively in eelgrass meadows near the mouth of the Jordan River, Maine and elsewhere along the New England coast. Underwater observations made while measuring tidal current profiles showed a dramatic waving response of eelgrass which produces up to a 50% variability in the midwater current speeds.

Because of the upper portion of eelgrass leaves moved through more of the water column per unit time while undulating in high current speeds than during times of low current speeds, we hypothesize that this mechanism resulted in the greater recruitment occurring on upper portions of the blades than on the lower portions. The distal tips were more likely to encounter larvae. We suggest that seagrass waving may affect hydrodynamical conditions above the canopy such that greater larval numbers are directed into the eelgrass bed than would arrive by other settlement mechanisms. Our hypothesis has major implications for larval recruitment in both field and flume studies.

**EFFECT OF AERIAL EXPOSURE OF *CRASSOSTREA RHIZOPHORAE* SPAT ON GROWTH AND SURVIVAL DURING GROW OUT.** Gary F. Newkirk\* and K. Richards, Biology Department, Dalhousie University, Halifax, N.S., Canada B3H 4J1 and Oyster Culture Project, Ministry of Agriculture, Kingston 7, Jamaica.

The mangrove oyster, *Crassostrea rhizophorae*, occurs naturally in the intertidal zone in the Caribbean. In Cuba culture of this species has been intertidal but in Jamaica subtidal culture has been developing. There is evidence to suggest that *C. rhizophorae* is physiologically adapted to the intertidal and that growth and survival is enhanced by aerial exposure. Recently spat collection in Jamaica has changed from intertidal collection to subtidal collection with a great increase in efficiency with respect to spat density per cultch. In this experiment spat exposed to different periods of intertidal exposure (4 hours per day, 1, 2, 4 and 6 days per fortnight) and different lengths of time on the spat racks (2 to 8 weeks) were monitored for growth and survival in subtidal grow out. There was an increase in mean size evident at 16 weeks as a result of increased aerial exposure during the month subsequent to set. Survival was not affected but yield of marketable oysters was also increased.

**TEMPORAL ANALYSIS OF THE RECRUIT-SETTLER RELATIONSHIP IN OYSTERS.** G. Curtis Roegner, Department of Oceanography, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

Hatchery-reared pediveliger larvae of the eastern oyster *Crassostrea virginica* (Gmelin) were used to evaluate temporal aspects of the relationship between the density of settlers and the

subsequent mortality of recruits. Controlled settlement, frequent photographic sampling of experimental substrates, and image analysis techniques allowed for a clear distinction between settlers and recruits over time. High initial mortality and variable responses to density attributable to the oyster growth form resulted in density-independent mortality patterns throughout the sample period. Despite this, the results indicate that estimating densities of settlers from densities of recruits using linear regression analysis is inappropriate with this species because of changes in the mortality rate (slope of the regression lines) with time.

## PARASITES AND DISEASE

**RECENT OBSERVATIONS ON THE SPORE STATE OF *HAPLOSPORIDIUM NELSONI* (=MSX) IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*.** Robert Barber,\* Sheila Kanaley, and Susan Ford, Haskin Shellfish Research Laboratory, P.O. Box 687, Port Norris, NJ 08349.

The spore stage of *Haplosporidium nelsoni* has been rarely reported (<0.01% of infected oysters) and a second host has been postulated. Recent intensive sampling of spat (<1 year) in Delaware Bay suggests that sporulation occurs regularly in this group. In May 1988, 30–35% of 1987 year class spat were infected with plasmodial stages of *H. nelsoni*. Infections became advanced and by June 20, 83% of spat with advanced infections contained spores. By July 5, prevalence was 10%, and the single advanced infection was found in sporulation. Between October 21 and December 2, 1/3 of July spat settlement were found with spores, representing 75% of advanced infections. In 1989, 6% of all spat were infected, three were advanced, and one found in late August had spores. In 1990, 10% of spat in June were infected and 75% of advanced infections were found with spores.

We also report finding operculated spores in the digestive tract lumina of fixed and stained sections of uninfected oysters, even in archived slides of oysters collected in 1958, during the original heavy epizootics of 1957–58 in Delaware Bay, and pre-dating the finding of *H. nelsoni* spores associated with plasmodial infections by two years. Preliminary evidence suggests that these spores are most prevalent in the months of July and September–October, coincident with, or just after peak periods of *H. nelsoni* spore production in infected spat. NJAES Publication No. K-32405-1-91.

**GULF OF MEXICO OYSTERS: HISTOPATHOLOGY, PARASITES, AND HEAVY METALS.** Stephanie A. Boyles,\* Eric N. Powell, and Robert Taylor, Dept. of Oceanogr., Texas A&M Univ., College Station, TX 77843; Julie Gauthier, Mar. Biomed. Inst., Univ. Texas Med. Branch, Galveston, TX 77550.

Oysters were collected in the Gulf of Mexico as part of the Status and Trends 1989 Year V (Mussel Watch) program at 63 sites from Brownsville, TX to the Florida Everglades. Each was analyzed for *Perkinsus marinus* prevalence and infection intensity

by the thioglycollate method, and examined histologically for the presence of digestive gland atrophy (DGA), number of brown bodies, and parasites including *Nematopsis prytherchi*, *N. ostreorum*, *Bucephalus* sp., and cestodes. *N. ostreorum* was widely distributed throughout the entire Gulf. *N. prytherchi* was abundant in oysters from most western Louisiana and Texas bays but the species was rare in the eastern Gulf. DGA was common in Gulf oysters: DGA had a Gulf-wide mean of 1.84 on a scale of 0 to 4 (0, no atrophy, to 4, heavily atrophied): 10 bays had means greater than 2.5. The abundance of brown bodies was higher in the southern Gulf (both eastern and western coasts) than in the northern Gulf. The body burdens of several heavy metals and polynuclear aromatic hydrocarbons (PAHs) were measured in the same oysters. The body burden of arsenic was significantly correlated with DGA, the number of brown bodies, and the abundance of *N. prytherchi* ( $P < 0.05$ ). The degree of DGA was also correlated with arsenic and copper ( $P < 0.05$ ), and the abundance of *N. prytherchi* was significantly related to selenium concentration ( $P < 0.05$ ). Combined with length, the histopathologies and parasites accounted for nearly 50% of the site-to-site variation in body burden for these heavy metals.

**CONCENTRATION OF *VIBRIO VULNIFICUS* IN OYSTERS, *CRASSOSTREA VIRGINICA*, GROWN IN PONDS WITH PACIFIC WHITE SHRIMP, *PENAEUS VANNAME*.** Victor G. Burrell, Jr.,\*<sup>1</sup> Jennifer Sample,<sup>2</sup> Coley Batay,<sup>1</sup> and M. Yvonne Bobo,<sup>1</sup> S.C. Marine Resources Research Institute,<sup>1</sup> NMFS Southeastern Fisheries Center, Charleston, SC<sup>2</sup>.

Shrimp farmers are interested in growing molluscs with shrimp in order to maximize profit and improve water quality. The presence of *Vibrio vulnificus*, a human pathogen, in southern waters and the tendency of *Vibrio* to be associated with chitonous shelled animals such as shrimp has raised public health questions about this practice. In order to address the question of *V. vulnificus* in oysters and the association of *V. vulnificus* with fecal coliforms in a pond situation, oysters naturally infected with *V. vulnificus* were grown in trays in ponds stocked with Pacific white shrimp. At the end of 30 days no *V. vulnificus* was detected in the oysters in ponds, however, in controls placed in the Colleton River *V. vulnificus* in oysters had increased. No further infections were noted in pond oysters while in the river oysters *Vibrio* remained fairly constant. *Vibrio* infection did not appear to be correlated with levels of fecal coliform in oyster meats and water, nor with salinity and temperature.

**CHEMOTAXIS OF *MERCENARIA MERCENARIA* HEMOCYTES TO BACTERIA.** Lynda B. Fawcett\* and Marenas R. Tripp, School of Life and Health Sciences University of Delaware, Newark, DE 19716.

The internal defense system of molluscs consists of hemocytes that migrate to and phagocytose foreign material in tissues. The

goal of this research was to identify mechanisms responsible for migration of *Mercenaria mercenaria* hemocytes using as *in vitro* assay (modified Boyden chambers). Responses to both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, marine isolates) bacteria were measured. Hemocytes migrate to live bacteria and culture supernatant but not to heat killed bacteria. The attractant is heat stable (100°C) and dialyzable. Treatment of culture supernatant with protease reduces but does not abolish activity suggesting that at least one attractant present in the supernatant is proteinaceous. N-acetyl glucosamine, N-acetyl muramic acid (both major components of bacterial cell walls), and formyl-methionyl-valine (a vertebrate leukocyte attractant) were not attractive. Tissue extracts from gill and mantle were also ineffective in inducing migration of hemocytes. *M. mercenaria* hemocytes migrate towards bacteria only in a gradient of attractant suggesting true chemotaxis (directed migration) vs chemokinesis (increased non-specific motility). The results of these *in vitro* studies indicate that chemotaxis of *M. mercenaria* hemocytes to bacteria may account for large accumulations of hemocytes at sites of infection *in vivo*.

**THE INCIDENCE AND ELIMINATION OF VIBRIOS AND FECAL-BORNE BACTERIA FROM NORTHERN NEW ENGLAND OYSTERS.** Stephen H. Jones,\* Kathleen R. O'Neill, Thomas L. Howell, Richard Langan, Aaron B. Margolin, and D. Jay Grimes, University of New Hampshire, Durham, NH 03824; Spinney Creek Oyster Co., Eliot, ME 03903.

The Great Bay/Piscataqua River Estuary in New Hampshire and Maine has an abundant oyster resource. Spinney Creek Oyster Company (SCOC) of Eliot, Maine, operates a depuration facility and relay lagoons for oysters harvested from the Piscataqua River in Maine. Following our recent discovery of *Vibrio vulnificus* in the estuary, we were interested in determining factors that may influence its incidence and elimination from oysters. Oysters harvested from 9 sites in the estuary, depurated and relayed oysters, and water samples were analyzed for the presence of fecal coliforms (FC), *Escherichia coli*, *V. vulnificus*, and total vibrios (TV). A polymerase chain reaction-based detection method for *V. vulnificus* was also tested. Fecal indicators were always detected at varying levels in water and oysters throughout the estuary. *V. vulnificus* was detected consistently during July–October, 1989–90, in all of the major tributaries, but only twice in Great Bay (GB). *V. vulnificus* was not detected during November–June, and its incidence was closely correlated with temperature and salinity. Depuration and relaying at SCOC resulted in drastic reductions in levels of fecal coliforms and *E. coli* in oysters TV and *V. vulnificus* did not respond to depuration. Relaying oysters to SCOC lagoons for 7 days had no effect on TV, but decreased levels of FC and *V. vulnificus*. FC levels decreased within 1 week and *V. vulnificus* was eliminated after 4 weeks in oysters relayed from the Piscataqua River to GB. These results demonstrate the

differences in incidence and the potential for elimination from oysters between indigenous, estuarine and fecal-borne bacteria.

**RECENT DEVELOPMENTS IN THE ANALYSIS OF DSP TOXINS.** J. C. Marr,\* T. Hu, and L. McDowell, Fenwick Laboratories Limited, Suite 200, 5595 Fenwick St., Halifax, N.S., Canada B3H 4M2; A. S. W. Defreitas and S. Pleasance, (under contract from SCIEX, Inc., Thornhill, Ontario), M. A. Quilliam and J. L. C. Wright, Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford St., Halifax, N.S., Canada B3H 3Z1.

One group of marine toxins that is recognized as a threat in both Europe and Japan is the family of diarrhetic shellfish poisoning (DSP) toxins. Okadaic acid (OA) and a methylated homolog of OA known as dinophysistoxin-1, DTX-1, are the most common. It has been reported that these toxins are produced by a number of dinoflagellate species. One group, belonging to the genus *Dinophysis*, has recently been found in eastern Canadian waters. Currently no regulatory limits have been established for DSP toxins in Canada. However, before limits can be realistically applied, standards of the toxins must be available and methods to analyze them must be in place.

In 1989, a collaborative research project between Fenwick Laboratories and the National Research Council of Canada was established. The research has focussed on the development of improved methods for extraction and clean-up for the preparative isolation of DSP toxins and for the routine analysis of trace levels in shellfish tissue. A major effort has been devoted to the development of improved HPLC methods, including the application of LC/MS technology for identification of the toxins. This expertise was used during a recent incident of DSP in Nova Scotia where a local source of cultured mussels was shown to contain high levels of DTX-1.

**STRUGGLE FOR SURVIVAL—THE PACIFIC RAZOR CLAM AND DISEASE.** Donald D. Simons,\* and Dan L. Ayres, Washington State Department of Fisheries, 331 State Highway 12, Montesano, WA 98563.

The Pacific razor clam (*Siliqua patula* Dixon) has long been one of the most sought after shellfish resources in Washington State. Beginning in the late 1800's as a commercial fishery, razor clam digging evolved into a popular recreational fishery. By the late 1970's, over 300,000 clam diggers made almost a million user trips a year. This tremendous effort resulted in a noticeable decline in the harvestable populations on all coastal beaches. Management biologist attempted to balance the increasing effort with the decreasing stocks. Then in 1983, during routine population sampling, losses up to 95% of all razor clams were discovered. All clam digging was suspended as studies were undertaken to discover the cause of the losses. The dramatic decline of the clams was from a previously unknown gill parasite labeled NIX (Nuclear Inclusion Unknown). The discovery of the disease and

its effect on the razor clam resource has permanently altered previous methods of management. This article outlines the changes and their effects on not only the resource but also on the users and local economy.

**HEMOLYMPH SERA FROM SARCOMATOUS AND HEALTHY SOFTSHELL CLAMS (*MYA ARENARIA* L.): DIFFERENT BIOCHEMICAL AND FUNCTIONAL MILIEUS.** Inke M. Sunila,\* and Christopher F. Dungan, Maryland DNR, Cooperative, Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654.

Serum-sarcoma cell interactions enhance transmission of sarcoma in the soft shell clam (*Mya arenaria* L.). Injection of sarcoma hemolymph (sarcoma cells and serum) results in disease transmission in recipient clams one month post injection. If sarcoma cells are isolated and administered in artificial sea water, in serum from healthy clams, in heat-treated sarcoma serum or in sarcoma serum digested with proteolytic enzymes, there is a similar lag period in all these groups. This suggests that tumor promoting factors in serum are heat sensitive proteins. Hemolymph samples from terminal stage or healthy clams, and repeated samples from clams with progressing sarcoma were collected in order to measure quantitative and qualitative differences in serum proteins. Total serum protein concentrations were significantly higher (1.7×) and more variable in clams at terminal stage of sarcoma than in healthy clams. The amount of serum proteins in diseased clams increased with time. According to SDS PAGE, different protein bands with more variability distinguished terminal stage clams from healthy clams.

We propose that some proteins present in the serum of healthy clams, but not in that of the sarcomatous clams, represent proteins originating from hemocytes (lysosomal enzymes, hemolysins and agglutinins), the lack of which explain, in part, impaired defense mechanisms of sarcomatous clams. We further propose that some proteins present in the serum of sarcomatous clams, but not in the healthy clams, represent proteins originating from sarcoma cells (growth factors, products of oncogenes), the functions of which might include mitogenic activity towards sarcoma cells and cytotoxic activity towards healthy hemocytes.

**DEFENSE MECHANISMS OF *GEUKENSIA DEMISSA*.** M. R. Tripp and K. Hackett, School of Life and Health Sciences, University of Delaware, Newark, DE 19716.

The ribbed mussel, *Geukensia demissa*, is not of commercial importance but is an important inhabitant of intertidal marshes. This is a preliminary report on its defense mechanisms. Microscopic examination shows hemolymph to contain granular and agranular amoebocytes, fibroblast-like cells and a few cells with distinctive inclusions. The most prominent cell is a distinctive granulocyte that readily phagocytoses red blood cells, yeast and bacteria. Hemolymph is not required for phagocytosis even though it contains lectins that agglutinate various biological par-

ticles. Cross-absorption experiments suggest that a complex family of lectins exists in normal hemolymph. In addition, an hemolysin of broad specificity is present in normal hemolymph. The importance of these factors for defense of *G. demissa* tissues will be discussed.

## OYSTERS

**EFFECTS OF LOW OXYGEN ON SURVIVAL AND GROWTH OF TWO SIZES OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* IN CHESAPEAKE BAY.** George R. Abbe,\* Richard W. Osman, and Denise L. Breitburg, The Academy of Natural Sciences, Benedict Estuarine Research Laboratory, Benedict, MD 20612.

Some of the large decrease in oyster production in Chesapeake Bay over the last few decades has been due to habitat loss from anoxic or near anoxic water spreading from deep areas toward shore during summer. Older oysters are able to withstand periodic low-oxygen conditions, but newly set oysters may not have this ability. To investigate the effects of oxygen-depleted water on various sizes, two age classes of oysters were placed in the field during 1989 and 1990 in water depths of 2, 5, and 9 m. Frequency and duration of low oxygen episodes during this time increased with depth. In each of three experiments 100 cm<sup>2</sup> panels, each with at least 100 spat (1- to 3-days old), were suspended 0.5 m above the bottom. Panels were retrieved after 1, 2, and 4 weeks, and surviving spat were counted and measured; panels were retrieved monthly thereafter. Trays of 1-year-old oysters were also deployed at the same sites with some 0.5 m off bottom and some directly on bottom; these oysters were measured monthly. After 1 month all oysters were photographed and growth was determined by analysis of digitized photographs.

During the first week at 2, 5, and 9 m, mean survivorship of spat was 36, 35, and 31%, respectively, and during the second week it was 56, 33, and 26%. Mean sizes after 1 week at 2, 5, and 9 m were 1.8, 1.5, and 1.0 mm, respectively, and after 2 weeks they were 4.8, 4.0, and 2.6 mm. Almost no mortality occurred among 1-year-old oysters, and growth differences were much less evident than for spat, but significantly lower meat conditions at the 9-m site indicated that they were also adversely affected by low-oxygen water.

**APPLICATION OF AN IMMUNOLOGICAL PROBE IN MEASURING INSTANTANEOUS REPRODUCTIVE RATE OF FEMALE EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*.** K.-S. Choi and E. N. Powell, Dept. Oceanogr.; D. H. Lewis, Dept. Veterinary Pathobiology, Texas A&M Univ., College Station, TX 77843; S. M. Ray, Dept. Marine Biology, Texas A&M Univ. at Galveston, Galveston, TX 77550.

A radio-immunological precipitation assay using a rabbit anti-oyster egg IgG as the primary antibody and *Staphylococcus*

*aureus* (Cowan I strain) cell suspension (Protein A) as an antibody adsorbent was developed for measuring instantaneous reproductive rate of oysters. Oysters were injected with <sup>14</sup>C-leucine via the adductor muscle and incubated in situ for 1 to 30 hr. The gonadal material was separated from other somatic tissues and homogenized in phosphate-buffered saline using a glass syringe homogenizer and an ultrasonicator. The quantity of gonadal protein was measured by an immunological ring diffusion test using rabbit anti-oyster egg serum in agarose gel coated on a glass plate. <sup>14</sup>C-labeled gonadal protein was precipitated from the homogenate using rabbit anti-oyster egg IgG. The antigen-antibody complex was further purified using Staphylococcal Protein A. Specific activity of <sup>14</sup>C-leucine was determined after separation on an amino acid analyzer. <sup>14</sup>C-leucine incorporation reached a maximum between 5 and 10 hr after injection. Between 20 and 30 hr, some loss of incorporated <sup>14</sup>C-leucine was observed. Instantaneous reproductive rates determined from the radio-immunological precipitation were found to be highest during August (of the months Aug.–Jan.) when oysters are actively investing their resources into egg synthesis for the upcoming fall spawning peak.

**EFFECTS OF IMMERSION TIME AND TIDAL POSITION ON THE *IN SITU* GROWTH RATES OF A NATURALLY SETTLED BIVALVE.** Michael P. Crosby,\* Cynthia F. Roberts, and Paul D. Kenny, Baruch Marine Lab, University of South Carolina, Georgetown, SC.

If intertidal populations of suspension-feeding bivalves are able to maintain equal (or greater) rates of growth as their subtidal siblings, a) adaptive metabolic capacities must exist in the intertidal populations to compensate for shorter feeding periods or b) selective disadvantages, such as predation or disease, must exist in the subtidal populations. Studying rates of growth in inter- versus subtidal suspension-feeding sibling bivalve populations under the same environmental conditions (other than immersion time) will demonstrate if such adaptations or selective disadvantages exists for a given species. We examined the effects of immersion time on *in situ* growth rates (from metamorphosis to mid/late juvenile state) of juvenile oysters which metamorphosed naturally in tidal creeks of a South Carolina salt marsh. Although spat height (ht) increased at equal rates during this period when regressed with days measured, and ht of inter- and subtidal spat was not significantly different on the initial days of measurement, the intercept of the ht with days measured model was significantly greater for sub- than intertidal spat. In addition, the overall mean of subtidal spat ht was significantly greater than that for intertidal spat. These results are indicative of differential rates of growth within and between inter- and subtidal oysters. The critical point of differential daily growth rate (RATE<sub>c</sub>) between sub- and intertidal spat began immediately post-metamorphosis and continued until each population had been immersed for a total of ~850 h. Prior to ~850 h of immersion, subtidal spat grew at a

significantly greater  $RATE_c$  than intertidal populations, although subtidal spat  $RATE_c$  did not significantly increase with increasing total hours submerged ( $HRS_T$ ). The initially lower intertidal  $RATE_c$  did significantly increase with  $HRS_T$  until  $\sim 850 HRS_T$ , at which time  $RATE_c$  plateaued. At  $>850 HRS_T$ , sub- and intertidal  $RATE_c$  were no longer significantly different.

#### **SURVIVAL AND GROWTH OF EASTERN OYSTERS HELD IN TRAYS IN CORPUS CHRISTI BAY, TEXAS.**

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The eastern oyster (*Crassostrea virginica*) was commercially harvested from Corpus Christi Bay until 1959, after which most reefs were non-productive. Reasons for the demise of oyster populations in Corpus Christi Bay have been attributed in-part to persistent high salinities. The re-establishment of an eastern oyster fishery in Corpus Christi Bay may be possible by stocking oysters preadapted to conditions similar to those found in Corpus Christi Bay. Populations of eastern oysters in South Bay, Texas are reported to survive, spawn and achieve rapid growth in salinities  $>40‰$ . The purpose of this study was to compare growth and survival of Eastern oysters from South and Corpus Christi bays held in Corpus Christi Bay. Eastern oysters were collected from South and Corpus Christi bays and maintained in trays for 12 months. There were no significant differences in mean total length between bay systems. However South Bay oysters had a significantly higher growth rate and survival percentage suggesting they may be better adapted to prevailing conditions within the bay.

#### **A COMPARISON OF HEMOLYMPH TO EXTRAPALLIAL FLUID OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.** Andrew S. Mount\* and Alfred P. Wheeler, Clemson University, Department of Biological Sciences, Room 132 Long Hall, Clemson, SC 29631.

The total calcium content of pooled mantle fluid (shell liquor), hemolymph, and extrapallial fluid was determined from oysters collected from North Inlet, South Carolina. Total calcium values among the compartments were found to have seasonal variability. Free calcium levels, determined by specific ion electrode, revealed approximately 30% of the total extrapallial fluid calcium was bound.

Proteins and free amino acids in the hemolymph and extrapallial fluid of the oyster appear to have different roles in the physiology of the organism. Specifically, the extrapallial fluid proteins were found to be similar to soluble, shell matrix proteins that have been extracted from oyster shell.

The free amino acid content of the fluids differed significantly among hemolymph and extrapallial fluid. Free amino acids detected in the extrapallial fluid bound nominal amounts of calcium

at physiologically relevant concentrations of magnesium. It was concluded that the complement of free amino acids detected in the extrapallial fluid do not have a direct role in nucleation events by an equilibrium or transport mechanism and therefore have little to do directly with shell formation. It is possible that free amino acids in the fluid may act in concert with other organic substances (such as organic acids) to form coordination complexes that would favor the formation of calcite in association with an organic matrix at the ionic strength of seawater.

#### **OYSTER CULTURE IN BON SECOUR BAY, ALABAMA.**

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Oyster culture along the Gulf of Mexico coast has not progressed much beyond providing substrate for spat despite declining production from public waters. A pilot project was initiated to investigate the feasibility of culturing the eastern oyster (*Crassostrea virginica*) at three sites in Mobile Bay using three variations of off-bottom, mesh bag culture.

Hatchery produced, cultchless spat (1.6–5.4 millimeters (mm) shell height) were held first in concrete raceways and later in a fertilized pond. After 45 days (d) oysters in mesh bags were moved to Bon Secour Bay and placed on a 2 meter vertical rack (7 levels), a horizontal belt suspended between pilings or on a belt suspended under a raft. As the oysters grew they were restocked into larger mesh bags at lower densities. After 60 d at the initial site, a portion of the oysters was moved to each of two other sites.

In the Bay, oysters grew from a mean 14.6 mm  $\pm$  0.5 SE (n = 100) to 42.8 mm  $\pm$  1.5 SE (n = 100) during the period 30 August 1990 through 31 January 1991 (156 d). Oysters on the vertical rack at the middle and bottom levels were larger than the suspended belt oysters. Oysters from the rack top level were smaller (mean = 36.1 mm  $\pm$  1.6 SE, n = 33), than those from the middle (mean = 48.3 mm  $\pm$  2.0 SE, n = 33), or bottom (mean = 48.1 mm  $\pm$  1.7 SE, n = 33) level. Oysters were also smaller on the top level of racks at the other two sites. There do not appear to be any important differences in growth among the three sites at this time. Overall, the oysters have reached 60 percent of harvestable length (75 mm) with negligible mortality in the 201 d of nursery and bay culture.

#### **IN VITRO ASSESSMENT OF GROWTH RATE ON OYSTER TISSUES: THE EFFECTS OF VERTEBRATE GROWTH HORMONE AND INSULIN.** Kennedy T. Paynter, The Johns Hopkins University, 4800 Atwell Rd., Shady Side, MD 20764.

Molluscan growth appears to be regulated to some degree by a hormonal system. For instance, bovine growth hormone and insulin have both been shown to increase the growth rate of abalone

larvae, and rainbow trout growth hormone stimulated oyster growth at low concentrations in seawater. In order to study the cellular aspects of bivalve growth, a series of experiments were conducted to examine the effects of trout growth hormone and insulin on amino acid incorporation, thymidine incorporation and oxygen consumption in oyster tissues.

Trout growth hormone and insulin stimulated oxygen consumption in isolated oyster tissues. However, neither treatment stimulated amino acid or thymidine incorporation in a variety of incubation experiments. Many treatment regimes were tested in which dose and incubation time were manipulated using both whole animal and isolated tissues. None of the treatments resulted in significantly increased incorporation rates.

In an effort to understand these confounding results, incorporation rates were measured in oyster populations that were known to be growing at different rates by virtue of being cultured at different sites. Oysters cultured in Mobjack Bay, VA, grew nearly twice as fast as animals grown in the Wye River, MD, however, incorporation rates of amino acids or thymidine, measured under ambient conditions during periods of active growth, were not different between the two groups.

Our results suggest that the biochemistry of bivalve growth is complex and the assessment of growth in terms of protein or DNA production may not be accurate. The results will be discussed in the light of similar research performed in other laboratories and in mammals.

**MODELING OYSTER POPULATIONS: THE EFFECT OF DENSITY AND FOOD SUPPLY ON PRODUCTION.** Eric N. Powell, Department of Oceanography, Texas A&M University, College Station, TX 77843; Eileen E. Hofmann and John M. Klinck, Department of Oceanography, Old Dominion University, Norfolk, VA 23529.

A time-dependent model has been developed to assess the response of oyster populations to environmental variables and the importance of population density in optimizing yield under varying climatic and hydrologic conditions. Food supply in a typical Gulf of Mexico bay, Galveston Bay, would appear to be just adequate in most years to maintain a healthy, productive oyster population. Any decline in food supply, by reduced food content, lower current velocity, lower temperature or increased population density, dramatically reduces yield and can eliminate reproductive capacity. The effect of temperature on filtration rate exerts an overriding influence, establishing, much more so than respiration, the energy balance of the organism, whether or not the population grows and reproduces, and to what extent current velocity and food supply affect productivity. The effect of decreased food supply, for any reason, is felt more strongly at higher latitudes. Large adult oysters probably have a negative energy balance during the winter in colder climates, except under the most favor-

able of food supply conditions. By contrast, higher temperatures at lower latitudes reduces somatic growth and, consequently, yield because more of the yearly net productivity is expended in reproduction.

## VIDEO SESSIONS

**DIRECT OBSERVATIONS OF FEEDING IN *PLACOPECTEN MAGELLANICUS*.** Peter G. Beninger,\* Département de biologie, Université de Moncton, Moncton, N.B., Canada E1A 3E9, Evan J. Ward, Bruce A. MacDonald, and Raymond J. Thompson, Marine Sciences Research Laboratory, St. John's, N.F., Canada A1C 5S7.

The technique of endoscopic video observation was used to study dynamic feeding processes of the gill of *Placopecten magellanicus* under near-natural feeding conditions. Particle fate depended on the extent of ingestive or handling capacity saturation. Under low to medium particle concentrations, all particles were initially directed to the dorsal ciliated tracts of the gill arch and dorsal bends. At high particle concentrations or after prolonged exposure to the lower particle concentrations, rejection progressively increased. The ordinary filament plicae were responsible for the ventral movement of rejected particles, counter to and below the incoming pallial current maintained by the principal filament cilia. Particle behavior suggested a probable mechanism for the rejection process. As particle concentration or period of exposure to lower particle concentrations increased, particle entrapment in mucus became increasingly evident, both on the frontal (capture) surfaces and on the dorsal ciliated (transport) tracts of the gill. The mucus-particle masses only formed discrete strings of the ventral ciliated (rejection) tract. No selection against inorganic polymer particles was observed. Results are discussed in the context of proposed theories of bivalve suspension-feeding.

**SUSPENDED SCALLOP CULTURE IN JAPAN—A VIDEO SUMMARY.** Cyr Couturier, Biological Station, St. Andrews, N.B. Canada E0G 2X0.

Japan is a world leader in the cultivation of scallops. In recent years, there has been a tendency towards bottom culture for final growout although intermediate culture and some final growout steps are accomplished in suspended culture. The video (13 min) depicts recent practices in suspended scallop culture in Japan. Methods of seed collection, intermediate, and final growout (ear hanging) are described. Certain steps have been mechanized ("ear piercing," grading, washing) and are also shown, but for the most part the industry remains labour intensive. Underwater footage of scallops in culture is provided. Finally, the "suitability" of Japanese culture methods to the North American pectinid scene will be discussed, time permitting.

**TRACKING LOBSTER MOVEMENT USING ULTRASONIC TRANSMITTERS.** R. E. Duggan\* and J. D. Pringle, Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S., Canada B3J-2S7; D. M. Webber and R. K. O'Dor, Department of Biology, Dalhousie University, Halifax, N.S., Canada B3H-4J1.

Fourteen ovigerous female lobsters, caught in the area of Jeddore Harbour, Nova Scotia, in June and July 1988, were fitted with ultrasonic transmitters. Movements were determined by using a portable ultrasonic receiver and a directional hydrophone to periodically record positions as Loran-C coordinates. Lobsters were regularly recovered by scuba divers using a hand-held underwater ultrasonic receiver. For the first time it was observed that late stage ovigerous females tended to move to a common area for larval release and then to a shelter to molt. On two occasions large males were observed with a molting female.

A second project was carried out in the same area in 1989. An array of buoys, each containing an ultrasonic receiver, omnidirectional hydrophone, radio transceiver, power supply and an ultrasonic transmitter was used to continuously track lobsters fitted with ultrasonic transmitters. Signals received from the lobster borne transmitters were relayed to a shore based receiver interfaced with a computer. Computer calculations permitted an assessment of time differences in signals received from different buoys to record individual lobster positions. Continuous monitoring over five days revealed nonoverlapping movement patterns demonstrating territoriality and evidence that peak activity is associated with nocturnal low tides. One lobster was fitted with a temperature sensitive transmitter which allowed the monitoring of both temperature and movement.

**HIGH-SPEED VIDEO ANALYSIS OF PARTICLE CAPTURE AND SELECTION BY MOLLUSC LARVAE.** Scott M. Gallager,<sup>1</sup> C. J. Langdon,<sup>2</sup> L. Davis,<sup>1</sup> and D. Stoecker,<sup>1</sup>

<sup>1</sup>Woods Hole Oceanographic Institute, Woods Hole, MA 02543, <sup>2</sup>Oregon State University, Hatfield Marine Station, Newport, OR.

We are using high-speed video microscopy to study how larvae use cilia to capture food particles from suspension, and what physical and chemical characteristics of particles are important to capture efficiency and selection for ingestion. We now know that larvae feed effectively on very small particles, including bacteria, in addition to larger phytoplankton cells. The efficiency of particle capture by cilia interception is a function of the magnitude and polarity of the electrostatic charge on particles: charged particles are intercepted and pushed into the food groove more often than particles with no net charge. Particle charge, in turn, is a function of the ionic composition and the dissolved organic loading of seawater.

Once a particle has been captured and carried to the mouth, the probability that it will be ingested or rejected is a function of its surface hydrophobicity. The larva's rejection mechanism works

well on hydrophilic particles, and may detect and use the presence of organic materials as an index of nutritional quality. The capacity for particle discrimination is lost, however, if the particle's surface is hydrophobic. In this case, all particles are ingested regardless of their composition.

Results will be discussed in relation to the size and compositional spectra of particles available to suspension-feeding larvae. Video tapes documenting these results will be presented.

**LOBSTERS, CRABS, AND VIDEOTAPES.** Peter Lawton,\* Fisheries and Oceans, Biological Station, St. Andrews, N.B., E0G 2X0, Canada; Kevin Taylor, Taylor Consulting and Services, Box 61, St. Andrews, N.B. E0G 2X0.

Contemporary video technology facilitates the recording and analysis of animal behaviour, both in laboratory contexts and, increasingly, in the marine environment. This video presentation reviews material from several laboratory studies in which the time budgets, and in particular the foraging behaviours, of decapod crustaceans were constructed using time-lapse video recording techniques.

With the advent of the 8 mm video format, compact camcorder systems, and low cost underwater housings, video monitoring of animal behaviour in the marine environment is now more accessible. A final video segment reveals how mature berried lobsters use shallow water areas off Grand Manan, Canada, and illustrates the diving survey approaches described in more detail by Lawton and Robichaud (in a separate contribution to these proceedings).

**A TIME-LAPSE VIDEO BENTHIC MONITORING (TLBM) DEVICE TO ESTIMATE FEEDING ACTIVITY IN MUSSELS: INITIAL FIELD RESULTS.** Carter R. Newell, Great Eastern Mussel Farms, Inc., Tenants Harbor, ME 04860 and Scott M. Gallager, Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

An instrument was developed to measure, *in situ*, the effects of particle concentration on feeding activity in undisturbed mussels. The tripod-mounted instrument uses time-lapse video technology to monitor shell gape of mussels for periods exceeding two weeks. Video tapes are analyzed field-by-field, either manually or by an automated video processor, to obtain shell gape of up to 30 mussels at intervals of one min.

In the laboratory, mussel shell gape was calibrated to filtration rate by measuring the decrease in chlorophyll with time following pulsed additions of cultured algae to filtered seawater in a running flume. In a field experiment, current speed, direction, particulate organic matter (POM), chlorophyll a, and filtration rate of mussels were measured simultaneously and correlated with shell gape of mussels recorded with the TLBM.

Results indicate a positive correlation between mussel shell gape and maximum concentrations of POM and chlorophyll a on the cbb tide, and a potential particle concentration threshold for

the initiation of feeding by mussels. Use of the TLBM to monitor predation, locomotory activity, and spawning events of benthic fauna is discussed.

**A VIDEO ASSESSMENT OF A LARGE MORTALITY EVENT IN A POPULATION OF THE SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*, IN THE BAY OF FUNDY, CANADA.** Shawn M. C. Robinson,\* James D. Martin, and Ross A. Chandler, Dept. Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0, Canada; G. Jay Parsons, Dept. Zoology, Univ., Guelph, Ont. N1G 2W1.

There was a large recruitment pulse in 1983 and 1984 in the Bay of Fundy to populations of the sea scallop, *Placopecten magellanicus*. The change in scallop densities was particularly noticeable in the Cape Spencer area off Saint John, New Brunswick and an intensive fishery quickly developed in 1988. However, reports of high mortality rates (i.e. clappers) occurred in the fall of 1989 and throughout 1990. In response to this, an ROV survey of the area was done in August 1989 to document the occurrence of both live scallops and 'clappers' on the bottom. A more traditional survey was also done in the same area a month later using standard Digby scallop drags to sample the population. This video will present footage of the ROV survey, some shots of scallop drags under fishing conditions, and how the video assessment compared to the traditional survey techniques.

**THE STUDY OF THE NIX DISEASE AND THE PACIFIC RAZOR CLAM.** Donald D. Simons, Washington State Department of Fisheries, 331 State Highway 12, Montesano, WA 98563.

The Pacific razor clam (*Siliqua patula* Dixon) has long been one of the most sought after shellfish resources in Washington State. Beginning in the late 1800's as a commercial industry, razor clam digging evolved into a popular recreational fishery. By the late 1970's, over 300,000 clam diggers made almost a million user trips a year. Then in 1983, during routine population sampling, losses up to 95% of all razor clams in Washington state were discovered. The dramatic decline of the clams was from a previously unknown gill parasite labeled NIX (Nuclear Inclusion Unknown). This video outlines studies that began in July 1990 to examine not only the disease NIX, but also razor clam population dynamics, genetics, aging of clams and other research.

**WASHINGTON RAZOR CLAMS—DIGGING, CLEANING, "CONSERVING."** Donald D. Simons\* and Alan D. Rammer, Washington State Department of Fisheries, 331 State Highway 12, Montesano, WA 98563.

The Pacific razor clam (*Siliqua patula* Dixon) has long been one of the most sought after shellfish resources in Washington State. Increasing effort and decreasing stocks are a constant concern for fishery managers. In 1979, legislation was passed to provide additional funding for increased enforcement, enhancement

and public education regarding the razor clam resource through a razor clamming license. One educational effort was to provide information to the clam digger through the use of a video, on how to properly dig for, clean and utilize the razor clam resource. Improper usage has led to wastage of millions of clams each year. This video has been shown to hundreds of thousands of viewers. It is a key component of our education program which plays an important role in the management of this species.

**DIRECT OBSERVATIONS OF FEEDING STRUCTURES AND MECHANISMS IN BIVALVE MOLLUSCS USING ENDOSCOPIC EXAMINATION AND VIDEO IMAGE ANALYSIS.** J. Evan Ward,\* Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, N.F., Canada, A1C 5S7; Peter G. Beninger, Département de biologie, Université de Moncton, Moncton, N.B., Canada E1A 3E9; Bruce A. MacDonald and Ray J. Thompson, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, N.F., Canada A1C 5S7; Roger J. E. Newell, Horn Point Environmental Laboratories, University of Maryland, Cambridge, MD, USA 21613.

A new technique is described for observing the structures and mechanisms of feeding in bivalves using endoscopic examination and video image analysis. This method permits direct *in vivo* observations of whole, intact structures of relatively undisturbed specimens. Pallial organ activity can be recorded for future observations and analysis.

Using this technique we examined four bivalve species: *Mya arenaria*, *Mytilus edulis*, *Placopecten magellanicus*, and *Crasostrea virginica*. Particle interception by the gills and transport of material to the palps was observed, and velocities of particles moving along gill filaments, and in dorsal and ventral ciliated tracts were determined. Differences in the extent to which material in food tracts was bound in mucus will be discussed. Advances in electronics have made the endoscope-video-analysis system an efficient and affordable technique which should prove valuable in elucidating mechanisms of feeding, and in testing hypotheses concerning the physical and chemical factors that affect pallial organ function.

## LOBSTER BIOLOGY AND ECOLOGY

**JUVENILE *HOMARUS AMERICANUS* STUDIES FROM MCNUTT ISLAND, NOVA SCOTIA.** Alan Campbell,\* Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6.

A 4.5 ha study area off McNutt Island, Shelburne, Nova Scotia, was surveyed annually during 1981–87. The substrate of the area had small pebbles interspersed with large boulders. The plant community changed from mostly crustose algae in the early 1980s to an increasing brown algal biomass during 1984–87. Although lobster densities fluctuated from 0.1 to 0.3 per m<sup>2</sup>, the

mean annual carapace length increased (29 to 47 mm CL) probably due to growth and immigration. The rock crab, *Cancer irroratus*, was generally more abundant than *H. americanus* and the jonah crab, *C. borealis*, but there was a higher biomass per m<sup>2</sup> of lobster than either of the two crab species.

**ESTIMATION OF RECENT GROWTH OF FIELD-CAUGHT POSTLARVAL LOBSTERS FROM RNA:DNA RATIOS.** J. Stanley Cobb,\* Marie A. Juinio, and David A. Bengtson, Department of Zoology, University of Rhode Island, Kingston, RI 02881.

Growth rates of postlarval (= stage IV) lobsters captured in the field were estimated from RNA:DNA ratios and average sea surface temperatures. Postlarvae (N = 358) in molt stages C and D<sub>0</sub> were collected at two sites in Block Island Sound during the summers of 1988–1990. Earlier laboratory observations (Junio et al., in preparation) on the relationship between nucleic acid ratios, temperature and mean growth rate (measured as mg protein day<sup>-1</sup>) allowed us to develop the equation: **Growth** =  $-.778 + .268 \text{ RNA:DNA ratio} + .01 \text{ Temperature}$ , which explained 86% of the observed variability in mean protein growth at molt stages C and D<sub>0</sub> for postlarvae reared at 18 and 21°C. This equation was used to estimate the recent growth rates of the field-captured postlarvae.

The mean protein growth per day in 1989 and 1990 was significantly higher in June than in July (p < .0002) suggesting that postlarval lobsters grew better earlier in the summer than later in the summer. There was no significant difference in mean protein growth between June of 1989 and 1990 but there was a significant difference between mean protein growth in July (p = .018). Mean protein growth in July of 1988 (.49 mg day<sup>-1</sup>) was significantly greater than that in July of 1989 (.39 mg day<sup>-1</sup>) but neither were significantly different from the mean protein growth in July of 1990 (.43 mg day<sup>-1</sup>). In all three years, the incidence of poorly nourished postlarval lobsters (i.e. < .20 mg day<sup>-1</sup>) was less than 5% of the total samples for each year. There was only one postlarva collected in 1990 which indicated a starved condition. There was no correlation between the estimated growth rates and postlarval abundances in Block Island Sound during these three years suggesting that food limitation is not a significant factor contributing to the interannual variability of postlarval recruitment in the area.

**COURTSHIP AND CHEMICAL SIGNALS IN THE AMERICAN LOBSTER.** D. F. Cowan, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

Behavioral experiments reveal that a complex system of primer and releaser pheromones may be involved in lobster courtship. It is widely accepted that female lobsters produce releaser pheromones at or around the time of ecdysis. These pheromones have been variously described as sex attractants, substances that reduce male aggression and initiate courtship, or as signals necessary in pair formation.

The male antennule has been cited as the organ for pheromone reception. However, when male antennules were removed, normal courtship behavior persisted. In contrast, when female antennules were removed aberrant behaviors resulted including unsuccessful couplings and death. Based on these results, male releaser pheromones serving to attract females and facilitate pair formation are more likely than vice versa. The role of female pheromones must be reassessed.

Female lobsters stagger their molts throughout the breeding season in order to pair bond and mate with the dominant male. The timing of female molts in the absence of males and in various sex ratios lend support to the primer pheromone hypothesis, which states that female odors inhibit and male odors accelerate molting by regulating the functioning of internal hormonal systems.

Chemical communication is obviously important to *Homarus americanus*, but we are only beginning to understand how releaser pheromones may function in courtship and primer pheromones in the regulation of reproductive behavior.

**EGG PRODUCTION AND RECRUITMENT IN A NEW-FOUNDLAND LOBSTER POPULATION: IS THERE A RELATIONSHIP?** G. P. Ennis, Science Branch, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, N.F. A1C 5X1.

From 1975, tagging, biological sampling, fishery monitoring and plankton sampling of larvae has been carried out annually in a localized lobster population on the southeast coast of Newfoundland. A 15-year series of estimates of annual standing stock, recruitment, egg production, and larval abundance indices is presented.

Standing stock varied from  $15.0 \times 10^3$  to  $26.6 \times 10^3$  lobsters of which 67.3 to 85.5% were recruits. Egg production varied from  $5.7 \times 10^6$  to  $86.8 \times 10^6$  eggs, and the mean number of stage I larvae per tow from 0 to 88. Variation in egg production and relative abundance of stage I larvae generally coincided although there were some inconsistencies. Assuming a 9-year lag between egg production and subsequent recruitment to the standing stock, the 6-year overlap in the data series indicates increasing recruitment with decreasing egg production. This result is inconsistent with the belief that recruitment in lobster populations is limited by low egg production and suggests the possibility of a dome-shaped relationship between spawning stock and recruitment near the low end of the potential egg production range.

**OFFSHORE STUDIES OF LARVAL LOBSTERS (*HO-MARUS AMERICANUS*) IN THE GEORGES AND BROWNS BANKS REGION.** Gareth C. Harding,\* John D. Pringle, Kenneth F. Drinkwater, Angus J. Fraser, Ian R. Perry, and Peter W. Vass, Habitat Ecology Division, Biological Sciences Branch, Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2.

Our geographic coverage of the region between the Canadian section of Georges Bank and Browns Bank illustrates that stage I

lobster are released on the banks and together with stage II larvae occur primarily on the banks. Stage III and IV lobster were collected both on and off Georges Bank and at times stage IVs appear more abundant off the bank. The higher lipid index, triacylglycerol/sterol ratio, measured in stage IV lobster off the bank, in both August 1988, and July 1989, is interpreted to indicate better health.

Loran C drifters drogued at 10 m depth travelled up to 30 km/day off the northern edge of Georges Bank. Lobster larvae were sampled with a Tucker trawl at least three times over a two-day period while following three drifters off and three drifters on the Bank. The lobster stage composition and abundance did not change significantly around the three off-Bank drifters. The lobster population on the Bank, with its predominance of stages I and II and their more pronounced daily vertical migration must follow the track of drifters drogued at 10 m depth less closely than the more surface living stage III and IV lobster located off the Bank.

**SEX RATIO DIFFERENCES BETWEEN ESTUARINE AND COASTAL LOBSTER POPULATIONS.** W. H. Howell and W. H. Watson, III, Zoology Department, University of New Hampshire, Durham, NJ 03824.

We are involved in a long-term investigation of the spatial and temporal distribution of juvenile and adult lobsters in Great Bay Estuary, N.H. One aspect of the study involves sampling the subadult and adult population, using standard traps, at a number of study sites ranging from the coast to the center of Great Bay, 14 miles inland. Smaller lobsters were sampled using traps covered with 1/2 inch mesh and by N.H. Fish and Game divers. The sex ratio data reported here is based on 1,736 animals captured and measured in 1989 and 5,186 in 1990.

One of the most interesting, and consistent patterns to emerge from this study is that the population in the upper estuary is dominated by males. In 1989, the ratio of males to females at the Nannie Island study site, farthest from the coast, was 8.6:1 and in 1990 it was 4.5:1. As one moves from this site toward the coast, the sex ratio gradually changes, approaching the more typical 1:1 ratio.

The sex ratio at each sampling site also changes during the course of the year. In the spring, most estuarine locations are dominated by males, with few females appearing in traps until late June. During the remainder of the year, there are still more males than females, but the ratio remains relatively constant.

In the estuary the sex ratio also differs between size classes. There are about equal numbers of males and females in the smaller size classes. However, in larger animals the ratio of males to females gradually increases from 1.5:1 (70–80 mm CL animals), to 12:1 in animals larger than 90 mm CL. In contrast, all size classes of animals collected in coastal waters had sex ratios approaching 1:1. Currently, our hypothesis is that differences in the migratory behavior of mature male and female lobsters gives rise to the observed sex ratio differences between coastal and es-

tuarine lobster populations. This research was supported in part by a grant from NOAA (Sea Grant).

**DEVELOPMENT AND DISTRIBUTION OF COASTAL PLANKTONIC LOBSTERS (*HOMARUS AMERICANUS*) IN THE WESTERN GULF OF MAINE.** Lewis S. Inceze\* and Terri Ainaire, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575.

Larval and postlarval (PL) lobsters were collected with neuston samplers for two seasons (July–September 1989–1990) in a coastal embayment, along outer coastal shorelines, around coastal islands and at "offshore" sites up to 15 km from shore and >100 m deep. Data on larval stages I–III were used to assess development of the larval population and make comparisons of relative abundance nearshore vs. offshore; they were not used to estimate absolute abundance because larvae occur mostly below the neuston layer. The progress of development in the field followed closely the predictions of laboratory work at corresponding sea water temperatures; distributions indicated that most larvae originate from outside the coastal bay. PLs, which are mostly neustonic, were sampled at most sites when they were abundant from early to middle August. No consistent nearshore vs. offshore trend in abundance was seen, and PLs were found at all sites within the coastal bay. The development of PLs was followed through intermolt stages and was consistent with rates of change predicted from laboratory work. Medium- and small-scale distribution patterns and the role of advection in PL supply to shallow water recruitment sites are discussed.

**A FIELD COMPARISON OF THE RECAPTURE RATES OF POLYETHYLENE STREAMER AND MODIFIED SPHYRION TAGS THROUGH MOLTING OF LOBSTERS (*HOMARUS AMERICANUS*).** A. Wade Landsburg,\* Department of Fisheries & Oceans, Invertebrates Division, Science Branch, Gulf Region, P.O. Box 5030, Moncton, N.B. E1C 9B6.

This study compares the efficiency of modified sphyron and polyethylene streamer tags. Modified sphyron tags are considered the standard for movement and growth studies of the American lobster (*Homarus americanus*). The polyethylene streamer tag was initially developed for tagging of shrimp species and further adapted to use on crustacean species but has never been reported to be used on *Homarus americanus*. One thousand eight hundred thirty-four (1,834) lobsters were tagged during the period July 3–7, 1989 in the Malpeque Bay, PEI area using polyethylene streamer tags (905) and modified sphyron tags (929). Tagged lobsters were recaptured during the commercial fishery May 1 to June 30, 1990. A high percentage (72.9%) of recaptured lobsters had molted during liberty as molting periods extend from late June to mid-September. Recapture rates of lobsters tagged and released using modified sphyron tags and polyethylene streamer tags differed significantly (19.2 and 43.8%, respectively). Possible differences considered include initial tag loss, tag induced mortality, loss during ecdysis and visibility of tags to fishermen at recapture.

**SUSPENSION FEEDING BY EARLY JUVENILE AMERICAN LOBSTERS, *HOMARUS AMERICANUS*. Kari L. Lavalli,\*** B.U. Marine Programs, M.B.L., Woods Hole, MA 02540.

Evidence to date suggests that early juvenile (<one year old) American lobsters are highly vulnerable to predation by benthic fishes and crabs. Remaining shelter-bound reduces the predation threat to some degree, but then food acquisition becomes problematic. The ability to suspension feed would allow lobsters to use a shelter-based food supply of plankton until such a size that they were less susceptible to predators. This ability was investigated using a laser backlighting system (as described in Strickler, 1985) and tethered Stage V and VI lobsters.

Lobsters use the exopodite current of the maxillipeds and the pleopod current to draw zooplankton into the region of the mouthparts. Once positioned by currents and movements of the third maxillipeds, the plankton is captured using one of several techniques, depending upon its size. If small, the prey is typically captured by a downward flexing of the third maxillipeds, such that the dactyl, propus, and carpus bend over the prey and sweep it back toward the second maxillipeds. This motion effectively traps the prey behind a mesh of serrate setae. Occasionally the prey is directly captured by the cuspidate setae of the second maxilliped dactyls. If the prey is large, it is either captured by the third maxillipeds alone (as described above), by the chelate walking legs alone, or by the walking legs working in tandem with the third maxillipeds. The claws are used very rarely to capture zooplankton and when used, the prey is transferred to the walking legs and then to the third maxillipeds.

Once captured, the prey is passed back to the second maxillipeds, which either accept or reject the item. If accepted, the item is stabbed by the cuspidate setae of the dactyls and passed back towards the esophagus. If rejected, the dactyl, propus, and carpus flex outward to push the item back into the exopodite current.

This feeding technique demonstrates that early juveniles can exploit different food sources from adolescent and adult lobsters and can remain shelter-bound for much of their vulnerable first year of life by suspension feeding.

**SHALLOW WATER SPAWNING AND MOLTING AREAS OF AMERICAN LOBSTERS, *HOMARUS AMERICANUS*, OFF GRAND MANAN, BAY OF FUNDY, CANADA. Peter Lawton\* and David A. Robichaud,** Fisheries and Oceans, Biological Station, St. Andrews, N.B., Canada E0G 2X0.

Between June and November, 1990, diving surveys in shallow (<20 m water depth) inshore areas around Grand Manan documented lobster population size and sex composition. Lobster density was recorded on 300 m<sup>2</sup> belt transects, with *in situ* measurement of carapace length, sex, molt status, and egg condition on berried lobsters.

Lobster populations occupying Seal Cove and Flagg Cove

differ in size and sex composition, and in the proportion of animals in soft shell condition. Different origins of lobsters participating in seasonal movements to these areas are suggested, as well as contrasting functions; Seal Cove being used for molting purposes, Flagg Cove representing a seasonal spawning area.

Surveying within Flagg Cove addressed fishermen's concern over potential effects on lobster habitat use of recently expanded salmon aquaculture activity. As compared with data from surveys undertaken in 1982, 1983, and 1989, a population displacement, away from the aquaculture site, is apparent in 1990.

As well as discussing the biological significance of these distribution patterns, a rationale is presented for the focus on spatial and temporal distribution, rather than on cause:effect linkages in addressing lobster:aquaculture interaction.

**MOLTING AND MOVEMENT OF LOBSTER, (*HOMARUS AMERICANUS*), IN AND ADJACENT TO MALPEQUE BAY, PRINCE EDWARD ISLAND, CANADA. Donald R. Maynard,\*** Department of Fisheries and Oceans, Invertebrates Division, Science Branch, Gulf Region, P.O. Box 5030, Moncton, N.B. E1C 9B6.

Malpeque Bay is a semi-enclosed bay of a maximum depth of 12 meters on the north coast of Prince Edward Island, with an active commercial lobster fishery. The lobster population inside and adjacent to the bay was investigated through tagging and molt monitoring to determine interactions between molting and movement trends. In 1989 molt stage monitoring of trap caught lobsters was conducted bi-monthly at six sites inside and outside the bay during the ice free period of May to November. A total of 3156 polyethylene streamer tags were recovered at the molt monitoring sites.

Four molting period peaks in the bay were observed on May 21st, June 8th, September 10th and October 22nd. This is the greatest number of consecutive lobster molt periods observed during one year. The highest percentage (60%) of advanced pre-molt and early post molt lobsters was observed during the June peak. Two molt periods outside the bay were observed to peak on September 24th and October 22nd.

Lobsters outside Malpeque Bay begin migrating into the bay in May. At the same time, some lobsters that over-winter in the bay begin to move out. Approximately 37% of the lobsters tagged in the bay remained as residents over a one year period. The relationship between lobster molting and migration is discussed in context with observed temperature.

**CHANGE IN LOBSTER SIZE AT MATURITY AMONG YEARS AND LOCATIONS. R. J. Miller and F. L. Watson,** Halifax Fisheries Research Laboratory, P.O. Box 550, Halifax, N.S. B3J 2S7.

Size at maturity strongly affects lobster egg production, and possibly year class strength. The proportion mature over a large

size range was measured in four ports in eastern Nova Scotia in four successive years. The biggest range in size at 50% maturity among years in one port was from 89 to 101 mm carapace length, and among ports in one year was as great as 73 to 101 mm carapace length. At the current minimum legal size and fishing intensity, eggs per recruit varied 2-fold among years at a single port, and 6-fold among ports within a year. Size at maturity and summer water temperatures were not well correlated.

**THE DEMOGRAPHIC CONSEQUENCES OF INTRASPECIFIC COMPETITION AMONG LOBSTERS (*HOMARUS AMERICANUS*).** Robert S. Steneck, Dept. of Oceanography, Darling Marine Center, Univ. of Maine, Walpole, ME 04573.

Shelter characteristics affect the carrying capacity of benthic habitats for lobsters 40–90 mm in carapace length. Field experiments were conducted over three successive summers in which shelter size and number were held constant but spacing was varied (at six shelter densities). Lobster population density increased significantly with increased shelter density. However, as population density increased the proportion of occupied shelters declined. Video observations revealed that agonistic encounters among lobsters >60 mm CL mediate direct intraspecific competition for space when shelters are close to one another. Thus, there is a measurable reduction in carrying capacity attributable to intraspecific competition. Surprisingly, as lobster densities increased, the proportion of large, competitively dominant, lobsters declined. The demographic result is that larger lobsters diffuse from regions of higher to regions of lower population density. This may effectively segregate the reproductive population from juveniles. I hypothesize that demographic diffusion is an adaptive solution for larger lobsters that are hormonally "hard-wired" to agonistically react to all conspecifics within a given range of detection.

**SCOTOPHASE REGULATION OF THE DIEL TIMING OF THE METAMORPHIC MOLT IN LARVAL AMERICAN LOBSTERS, *HOMARUS AMERICANUS*.** S. L. Waddy and D. E. Aiken, Dept. Fisheries and Oceans, Biological Station, St. Andrews, N.B., Canada E0G 2X0.

When larvae of the American lobster are reared communally in planktonkreisels at 20°C, the molt to fourth stage (metamorphic molt) occurs predominately (70–98%) during the scotophase. The diel timing of the molt is related to the timing of the scotophase. Larvae adjust the timing of their molt when the light/dark cycle is reversed and the scotophase is 12-h out of phase with that experienced during embryonic development and the molt to first stage. Diel timing of the molt does not vary significantly with changes in scotophase length (LD 14:10, 12:12 or 10:14) or time of year the experiments were conducted (January through August).

Results suggest that timing of the metamorphic molt is related to the onset of darkness ("lights-off"). LD 18:6 and 14:10 photoperiod regimes produced similar molting responses, despite the

difference in duration of the dark phase. Although the percentage molting in the dark was significantly different in the two groups (56 and 79%), similar numbers (78 and 79%) molted within 10 hours of "lights-off." In short scotophases (LD 18:6 and 21:3), 56 and 52% of the larvae molted within six hours of "lights-off." These results suggest there may be endogenous molting rhythm in lobster larvae.

**IMPLICATIONS FOR LOBSTER FISHERY ENHANCEMENT FROM NATURAL BENTHIC RECRUITMENT, HATCHERY-REARED 'BLUES,' AND EXPERIMENTAL COBBLES.** Richard A. Wahle, Program in Ecology and Evolutionary Biology, Brown University, Providence, RI 02912.

Attempts to artificially enhance the American lobster fishery have been limited by the difficulty of demonstrating their efficacy. Enhancement efforts have been aimed at boosting benthic recruitment by either releasing hatchery-reared post-larvae in the wild or by increasing the amount of suitable benthic habitat available to new recruits. To date, larval releases have been most popular, but the technique remains unproven because of the inability to track the fate of lobsters after release. Here I describe patterns of benthic recruitment of lobsters in New England which became the spring-board for field experiments on habitat enhancement and releases of hatchery-reared blue lobsters that I have been able to follow through time.

Diver operated airlift suction samples from cobble recruitment habitat at sites in the northeast Gulf of Maine to Narragansett Bay reveal fairly consistent benthic recruitment to outer coastal areas from Penobscot Bay, ME south. Recruitment was more sparse northeast of Penobscot Bay and in upper Narragansett Bay, but the extent to which larval supply or physiological constraints may limit recruitment in these areas is unclear. Nonetheless, identifying areas with and without consistent recruitment is the first step to determining where and what kind of enhancement may be warranted.

The habitat enhancement experiment has been in place since July 1989 and consists of an array of twenty-five 0.5 m<sup>2</sup> cobble plots on a featureless sand bottom in a cove in Maine. Lobsters (approx. 30–40 mm carapace length) travelling along the bottom almost immediately occupied the plots increasing population density by more than a factor of ten (to approx. 6/m<sup>2</sup>). In addition, post-larvae have settled in low densities.

Hatchery-reared blue lobsters were released in these plots in 1989 and 1990. Within a day or two of the release, population densities stabilized at about 1/5th the original number at 1–3 individuals/m<sup>2</sup>. An issue critical to the efficacy of this kind of enhancement will be to distinguish emigration losses from mortality losses. As blue lobsters from both releases continue to be found in the plots, I am accumulating previously unavailable data on the growth of early benthic phase lobsters in nature.

## BIVALVE GROWTH AND SURVIVAL

**EFFECTS OF INTRASPECIFIC DENSITY ON THE SURVIVAL OF *ARCTICA ISLANDICA* (L.) WITHIN FIELD ENCLOSURES LOCATED IN EASTERN MAINE, USA.** Brian F. Beal,\* and M. G. Kraus, Univ. of Maine at Machias, Machias, ME 04654.

In Maine, ocean quahogs, *Arctica islandica* (L.), support a small fishery in the easternmost part of the state centering around Machias Bay (Lat. 44°35'N; Long. 67°26'W). Approximately sixty boats ( $\leq 12$  m) operate from three coastal communities where, in 1989, their reported dockside landings reached \$2.4 million, an increase of 30% from the previous year. This fishery has reached second in commercial importance Downeast surpassing softshell clams and behind only lobsters. To provide shellfish managers with quantitative information about natural mortality rates, we designed a field study to test the importance of intraspecific density and predation on the fate of commercial-sized individuals.

To test the interaction of initial density and predation, we placed individually marked and measured animals ( $\bar{X}$  SL = 48.7 mm  $\pm$  0.07 SE; n = 2325) into each of three types of vinyl-coated mesh (12 mm aperture) 30.5 cm<sup>3</sup> enclosures (completely open, completely enclosed, and partially enclosed) at each of three stocking densities chosen to reflect natural conditions (130/m<sup>2</sup>, 323/m<sup>2</sup>, and 645/m<sup>2</sup>). The experiment was conducted at a depth of 20 m and was initiated during a two week period in September 1985. Five replicates of each combination of density and cage type were employed and the experiment lasted one year. In addition, we tested the importance of transplant date and conducted a similar one year test in the same area from April 1986 to 1987.

Independent of density or caging treatment, annual survival of quahogs transplanted to field plots beginning in April 1986 was significantly higher (95.2%  $\pm$  1.86 SE; n = 21) than those placed into plots in September 1985 (89.3%  $\pm$  2.59 SE; n = 26; P = 0.03). The effects of density and caging on survivorship were consistent within and between the two experimental intervals (P = 0.95 and P = 0.46, respectively) as quahogs at the intermediate density of 323/m<sup>2</sup> survived significantly better (97.9%  $\pm$  2.21 SE; n = 15) than those at either lowest and highest densities (88.4%  $\pm$  2.13 SE; n = 32). No differences in survival rates were detected between animals originally added to open or partially enclosed plots (89.7%) which had a lower combined survivorship than those quahogs completely protected from predators (95.6%, P = 0.03). Although mortality was low, the major damage type observed was chipped and completely crushed valves. *In situ* predator inclusion tests revealed that the lobster, *Homarus americanus*, was the major predator of large quahogs.

To determine whether the influence of intraspecific density on survival rates observed during the two year-long experiments was

typical, we conducted similar density tests for two and three years, respectively. Beginning in September 1985, quahogs were added to open 30.5 cm<sup>3</sup> enclosures at each of the stocking densities referred to above. No differences in survivorship (P = 0.46) were observed between years (2 yrs = 87.6%  $\pm$  3.87 SE, n = 17; 3 yrs = 83.1%  $\pm$  5.48 SE, n = 16). Although there was a 17% difference in survival rate between animals stocked at 130/m<sup>2</sup> vs 645/m<sup>2</sup> (92.9% vs 75.8%) this effect was not significant (P = 0.08) but was consistent between years (P = 0.10). These results suggest that ocean quahogs effectively reach a size refuge from predation and that thinning, by removal from fishing or other means, should have no serious impact on individual survival rates during that part of the year when lobsters are not abundant.

**THE IMPORTANCE OF INITIAL SIZE AND DENSITY ON THE SURVIVAL AND GROWTH OF HATCHERY-REARED INDIVIDUALS OF *MYA ARENARIA* L.** Brian F. Beal\* and M. Gayle Kraus, University of Maine at Machias, 5 O'Brien Avenue, Machias, ME 04654.

During the period 1982 to 1989, softshell clam landings in Maine declined 65% from  $6.7 \times 10^6$  pounds of meat to  $2.4 \times 10^6$  pounds. Because traditional attempts to manage clamming habitat have historically produced inconsistent results, ten Downeast coastal communities have adopted an untraditional approach to increasing local productivity: public aquaculture. Beginning in 1987, a regional shellfish hatchery, located on Beals Island, has annually produced an average of ten million 8–12 mm juveniles of *Mya arenaria* for seeding on public-owned clam flats in an effort to enhance existing stocks. Because little published information exists concerning the fate of hatchery-reared *Mya* in the field, we conducted a series of manipulative studies to examine how initial clam size and stocking density affects survival and growth.

The first test was conducted from 16 Sept to 10 Dec 1989 at two mid-intertidal sites: Holmes Bay, Cutler and the Chandler River, Jonesboro. Three clam sizes were chosen: small ( $\bar{X}$  SL = 3.9 mm  $\pm$  0.08 SE; n = 37), intermediate ( $\bar{X}$  SL = 11.2 mm  $\pm$  0.16 SE; n = 36) and large ( $\bar{X}$  SL = 17.8 mm  $\pm$  0.26 SE; n = 48). Clams were added to 45 open field enclosures (15 cm diam  $\times$  15 cm deep) at a density of 660/m<sup>2</sup> at each site. The influence of initial size varied between sites (P < .05). At Cutler, intermediate and large individuals had a higher survival rate than small clams (92% vs. 81%; P = 0.02). At Jonesboro, 63% of small and intermediate size clams survived compared with 84% of the larger clams. The boring gastropod, *Lunatia heros*, accounted for an average 12.4% of all losses across all treatments at Jonesboro compared with <1% at Cutler. Relative growth rates ((final length – initial length)/final length) also differed between sites (P < .001). Individuals at Cutler grew twice as fast as those at Jonesboro while small *Mya* at both sites grew nearly 3 times faster than the larger ones.

In the second experiment, which was conducted at Cutler from

23 July 1990 to 16 September 1990, *Mya* of two sizes ( $\bar{X}$  SL = 8.7 mm  $\pm$  0.19 SE, n = 100;  $\bar{X}$  SL = 13.9 mm  $\pm$  0.21 SE, n = 100) at each of two densities (660/m<sup>2</sup> and 1320 m<sup>2</sup>) were added to a total of 176 open field enclosures. After one month, one-half of the enclosures were sampled. Survival rate of large clams (89%) was significantly higher (P < .001) than smaller clams (65%). Density had no influence on these rates (P = 0.55). By September, another 5% of the smaller clams were lost compared with an average of 15% for the larger; however, this latter loss rate was influenced by density (P < .05). Final survivorship for large clams at 660/m<sup>2</sup> was 83% versus 64% for those at 1320/m<sup>2</sup>. Seventy percent of the growth observed during the entire experiment occurred during the first month. By August, small clams had attained a mean size of 16.8 mm  $\pm$  0.11 SE (n = 513) and large clams were 22.4 mm  $\pm$  0.11 SE (n = 662). These rates were influenced by density as in both instances animals stocked at the higher density grew significantly faster than those at the lower density (+6.7% for small clams, P < .001; +2.4% for large clams, P = 0.04). By the September sampling, small clams had added another 3.6 mm in SL whereas large clams had added 3.0 mm. No density effects were observed on relative growth rate. These results suggest that communities participating in public stock enhancement programs wishing to maximize their investment, develop transplant strategies which take into account the effects of site, transplant time, clam size and stocking density.

**COMPLIANCE BEHAVIOR IN THE RHODE ISLAND QUAHOG FISHERY.** Casey E. Bean\* and Jon G. Sutinen, Department of Resource Economics, University of Rhode Island, Kingston, RI 02881.

Compliance behavior of Rhode Island shellfishermen has been successfully measured for the first time in the hardshell clam "quahog" fishery (*Mercenaria mercenaria*). Over 400 shellfishermen responded to a mail survey in July 1988 which gathered data on (1) respondent's contacts/experiences with the DEM's Division of Enforcement conservation officers, (2) their "perceptions" of violation activity in the industry, and (3) their perceptions of the effectiveness of enforcement's efforts. The goals of this research were to measure the extent of noncompliance in the fishery, and to test the effectiveness of a *proxy subject* methodology where fishermen are asked to describe illegal behavior not of themselves, but of anonymous fishermen.

Based on perceptions of the respondent's, it was estimated that in the 12 months prior to July 1988, an average of 13 percent of all commercial shellfishermen were responsible for taking 890,000 pounds (shellweight) of *uncertified clams* valued at \$1.5 million. These illegal landings represent five percent of the state's reported landings. The *typical violator* described was 32 years old, a full-time shellfisherman, and a fisherman for 10 years.

Two-stage ordinary least-squares estimation was used on the data set to estimate how violation activity is influenced by the

probability of being detected and convicted, demographic characteristics of the anonymous violator, their experiences with enforcement authorities, and the area of the Bay fished. Results indicate that enforcement efforts such as water patrols do affect the violation rate; however, most demographic factors are not significantly related to changes in violation rates among individuals.

A similar study performed two years earlier in the Massachusetts' lobster fishery provided unique comparisons of the enforcement programs. One finding was that Rhode Island shellfishermen were four times more likely to be detected fishing illegally and 100 times more likely to be caught and convicted than Massachusetts' lobstermen.

The study demonstrated the effectiveness of the methodology in measuring compliance behavior, and indicated to policy makers and the industry that enforcement efforts are perceived by shellfishermen to produce a deterrent to illegal fishing.

**PHYSIOLOGICAL ENERGETICS, GROWTH AND BIOMASS ALLOCATION OF MUSSELS ACROSS THE INTERTIDAL ZONE.** Francisco J. Borrero\* and T. Jerry Hiltbish, Department of Biological Sciences and Belle W. Baruch Institute, University of South Carolina, Columbia, SC 29208.

High intertidal populations of suspension feeding bivalves exhibit reductions in potential feeding time that result from reduced periods of inundation. Current data on behavior and feeding physiology of bivalve molluscs is not sufficient to assess whether individuals at high intertidal elevations exhibit compensatory mechanisms for reduced feeding time. Determinations of growth and physiological performance of populations of mid- and high intertidal mussels (*Guekensia demissa*) from salt marshes in South Carolina can be used to address this question.

High intertidal mussels have considerably lesser somatic and reproductive biomass, but maintain similar proportional allocation of biomass to reproductive tissues than mussels from the mid-intertidal zone. Reduced periods of inundation have a stronger effect on growth of soft tissue than on shell growth. No consistent differences in feeding, absorption or metabolic rates were observed among mid- and high intertidal mussels, during periods of submergence or aerial exposure. Estimations of net energy balance from physiological measurements suggest the absence of physiological compensations for reduced feeding time by high intertidal mussels. The energy budgets of these populations are dictated by extrinsic conditions that result from length of submergence.

**GEODUCK CLAM RESEARCH IN BRITISH COLUMBIA.** Alan Campbell\* and Donald J. Noakes, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6.

The geoduck clam, *Panopea abrupta* (Conrad), the largest and longest lived (up to 146 y) hiattellid in British Columbia, currently supports the region's most valuable invertebrate fishery. Research

on the population biology of geoducks has recently increased in support of fisheries management of this species in B.C. One study examining the annual growth rates of geoducks from Ladysmith Harbour has related growth with environmental changes. Acetate peels from cross sections of the right valve shell of each clam were made and age and growth were estimated from the number and distances between the annuli on the hinges. An average annual standardized growth index of geoducks was calculated for 1907–1980. Changes in growth were observed around 1919 and 1962. Intervention analysis was used to quantify changes in mean standardized growth for these two periods. Mean annual temperature increases around 1920 were coincident with an 8% increase in geoduck growth. A 27% decrease in growth occurred soon after 1960 when log booming and storage started in Ladysmith Harbour. Geoduck shells may be useful in relating changes in the marine environment over a 50–100 y period.

**GROWTH AND SURVIVORSHIP OF OCEAN QUAHOGS, *ARCTICA ISLANDICA* (LINNAEUS) IN AN INTERTIDAL MUDFLAT IN EASTERN MAINE.** M. Gayle Kraus and Brian F. Beal, Science/Math Div. Univ. of Maine at Machias, Machias, ME 04654; Louanne, McMartin, Biology Dept., Univ. of Southern Colorado, Pueblo, CO 81001.

Ocean quahogs have been assumed to be absent from intertidal regions because of their sensitivity to estuarine conditions. Based on the results of a salinity/temperature tolerance test, which is being presented separately, we designed a six month (April to October 1988) experiment to determine the feasibility of growing *A. islandica* in an intertidal mudflat. Five individually marked and measured juveniles (SL  $\bar{X}$  = 11.2 mm, SE  $\pm$  0.12; n = 445) were added to each of 45 experimental units (12.4 cm diam  $\times$  12.6 cm deep) at each of 2 tidal levels (low & mid). At both tidal heights, 15 replicates of each of three treatments were randomly arrayed: open enclosures to allow access by predators and units covered with one or two layers of 3.2 mm VEXAR mesh to exclude predators. After six months, only 8 of the 225 quahogs placed at midtide were alive. None had grown significantly. However, treatment had a significant ( $p > 0.001$ ) effect on survivorship at low tide. Only 2.2% of the quahogs were recovered alive in the open units and most of the pots contained small shell chips, consistent with damage created by crustaceans. 93.3% survived in the containers covered with one mesh, while only 62.2% survived in the double-mesh units. Quahogs at low tide exhibited a mean relative growth rate of 12.8% (final SL  $\bar{X}$  = 13.1 mm SE  $\pm$  0.43; n = 71), but some individuals, particularly those nearest the water, had a relative growth rate  $>30\%$ . Again treatment had a significant ( $p > 0.001$ ) effect. Relative growth was highest ( $\bar{X}$  = 19.7% SE  $\pm$  0.02; n = 42) for the units covered with a single mesh and lower ( $\bar{X}$  = 8.2% SE  $\pm$  0.02; n = 28) for those double meshed. No growth occurred in the units open to predators. Growth rates in this experiment did not approach those obtained in

laboratory-reared individuals, but the results confirm the need for a predator-free, food-rich environment to maximize growth as well as survivorship in juvenile ocean quahogs.

**GROWTH RATES OF *MERCENARIA MERCENARIA* IN PRINCE EDWARD ISLAND.** Thomas Landry\* and Thomas W. Sephton, Department of Fisheries and Oceans, Mollusc Aquaculture Section, P.O. Box 3050, Moncton, N.B. E1C 9B6.

Results from a preliminary survey on growth rates of quahaugs (*Mercenaria mercenaria*) from Pownal Bay and West River, P.E.I., reveal significant differences between those two sites. A reciprocal transfer experiment was used to determine if these different growth rates are due to genetical or environment differences.

A total of 120 quahaugs, ranging in size from 25 mm to 45 mm in length, were collected from each site, and tagged. Half (60) of these (quahaugs) were transplanted to the alternate site, while the remaining 60 were replanted at their original sites. Temperature, salinity, chlorophyll and seston were measured on a monthly basis. Growth and mortality levels were determined at the end of a four month period.

The growth increments of quahaugs in West River were 3.6 mm and 5.3 mm for quahaugs originating from Pownal Bay and West River respectively, compared to 0.7 mm and 1.0 mm in Pownal Bay. Mortality was higher in West River group at the West River site (31 dead animals) compared to an average of 6.3 from the three remaining groups. These differences will be discussed in view of the results of the environmental parameters.

**SUMMER MORTALITY OF CULTURED BLUE MUSSELS IN PRINCE EDWARD ISLAND, CANADA.** Thomas W. Sephton\* and Clair F. Bryan, Department of Fisheries and Oceans, Science Branch, Gulf Region, P.O. Box 5030, Moncton, N.B., Canada E1C 9B6.

A recurring problem that effects a large proportion of the cultured mussel industry in P.E.I. is the summer die-off of mussels (*Mytilus edulis*). Preliminary attempts by others to address this problem, which is believed to be associated with post-spawning stress, have been inconclusive.

A three year study is in progress to examine the influence of genetic and environmental factors on summer mortality of P.E.I. mussels using a reciprocal transplant experiment with 6 study sites (on mussel farms in New London Bay, Tracadie Bay, St. Peters Bay, Boughton River, Cardigan River, Murray River). Mortality (age, origin of seed), growth (shell) and animal condition (using carbohydrate as an index) are examined at seasonal intervals.

In the spring of 1990, a total of 18 pearl nets (3 from each study site, each containing 250 juvenile seed mussels) were placed at each location (total of 108 pearl nets and 27,000 mussels). En-

vironmental parameters (temperature, salinity, seston, chlorophyll, water currents) were monitored at all sites every 2 weeks during the ice free season and periodically during winter. In mid September, the entire experiment was sampled to determine rates of mortality and growth over the summer interval and the mussel densities standardized for the winter. There was an inverse relationship between mortality and growth of the different seed sources which was consistent among sites: mortality ranging from 2.5% to 30%, with the average interval growth increment ranging from 9.9 mm to 5.8 mm. Factors influencing mortality and growth are discussed. The water temperature data showed that this past summer was about 3°C warmer than that observed in the past 2 years. The hot, relatively dry summer conditions of the region in 1990 may explain some of the summer mussel mortalities reported from various locations in P.E.I. and N.B.

**EFFECTS OF SUBSTRATE MODIFICATION ON THE GROWTH AND SURVIVAL OF PLANTED MANILA CLAM SEED (*VENERUPIS JAPONICA*).** **Derrick R. Toba\*** and **Kenneth K. Chew**, University of Washington, School of Fisheries WH-10, Seattle, WA 98195; **Doug Thompson**, Washington Department of Fisheries, Point Whitney Shellfish Lab, 1000 Point Whitney Road, Brinnon, WA 98320.

In recent years, state-wide production of the Manila clam, *Venerupis japonica*, has increased so greatly that Washington is now the largest producer and exporter of Manila clams in the United States. Despite its rapid growth, the industry cannot meet market demand. Much of the optimal areas in Puget Sound are already under cultivation. One method to increase production is through increasing the amount of habitat by substrate modification.

A site at Bywater Bay, Hood Canal, was constructed to test whether the addition of crushed oyster shell to a previously gravelled plot would increase the growth and natural recruitment of Manila clams. The purpose of adding crushed oyster shell was to counter the effects of gravel compaction and increase the surface area for settlement of clam larvae. Three replicates of the following treatments were established on 2 × 4 meter plots: 1) control, no substrate alteration; 2) substrate rototilled to a 10.0 cm depth; 3) a 5.0 cm layer of crushed oyster shell rototilled to a 10.0 cm depth. Rototilling a 5.0 cm layer of crushed oyster shell to 10.0 cm would give a 50/50 ratio of shell to existing gravel. To each of the plots, 5 to 6 mm Manila clam seed were added at a density of 700 clams/m<sup>2</sup>. One half of each plot was covered with 7 × 14 mm predator exclusion netting. At four month intervals, five core samples on each half of the plot are randomly sampled to measure the growth and survival of the planted clam seed. Initial results have shown higher survival on the half protected with netting. Growth rate was highest on the plot in which crushed oyster shell was added and was lowest in the controls.

## FEEDING BY BIVALVES

**MODIFYING NURSERY TECHNIQUES FOR PEDAL BY FEEDING JUVENILE GEODUCKS (*PANOPEA ABRUPTA*) IN A SAND SUBSTRATE NURSERY.** **Lauran R. Cole** and **J. Harold Beattie**, Washington State Department of Fisheries Shellfish Laboratory, 1000 Point Whitney Road, Brinnon, WA 98320.

The Washington Department of Fisheries (WDF) facility at Point Whitney now produces 10 mm geoduck seed for reseeding commercially harvested plots. Although hatchery techniques yield consistent numbers of geoduck larvae, survival and quality of early juveniles during the nursery stage has been variable.

Post-larval geoducks are mobile, and feed with their active feet from benthic diatoms, detritus and bacteria for several weeks after the onset of metamorphosis. At the WDF nursery sites, post-larval geoducks grow in sand substrate raceways that pump water from a fertilized sea water pond as the only source of food. Experiments during 1990 used nursery microcosms to test survival and growth of plantigrade geoducks raised in pond water with wild algae, to those raised in filtered water with supplemental food. Supplemental food treatments included: 1) dried *Tetraselmis suecica* added in a slurry to the substrate, 2) dried *Tetraselmis* suspended in the water column, 3) live cultured *Chaetoceros muelleri* suspended in the water column, and 4) non-fed controls. Preliminary results show significant differences in growth between treatments with food available to the substrate and those with food added only to the water column.

**MUSSEL FEEDING SELECTIVITY BELOW THE PSEUDOFECES THRESHOLD: THE IMPORTANCE OF PARTICLE CONCENTRATION.** **Carter R. Newell,\*** Great Eastern Mussel Farms, Inc., Tenants Harbor, ME 04860; **Sandra E. Shumway**, Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, ME 04575.

In order to follow-up on earlier evidence of a feeding selectivity threshold of mussels (*Mytilus edulis*) feeding below the pseudofeces threshold on natural particle assemblages (Newell et al., 1989, JSR 8:187–196), feeding experiments were performed in winter of 1989 and fall of 1990 using the flow cytometer analyzing particles 3–50 microns in diameter. Diets were composed of natural seston with filtered seawater, mudflat silt or cultured algae added to result in ranges of food quality of 18–36 percent fluorescent particles at 7.6–10 million particles per liter, and 10–25 percent fluorescent particles at 16–22 million particles per liter. Regardless of the food quality, mussels were able to feed significantly faster on algae than on silt particles at low concentrations. However, at seston concentrations above 17 million particles per liter, mussel feeding rates were not significantly different with respect to algae or silt, regardless of the quality of the diet. Since this is just below the pseudofeces threshold, it appears that mussels may be able to select particle types both in dilute

suspensions (perhaps on the gills) and in dense suspensions (on the palps) but in the transition between these two concentrations they are indiscriminate feeders.

**STIMULATORY EFFECTS OF MICROALGAL CHEMICAL CUES ON THE CLEARANCE AND INGESTION RATES OF *PLACOPECTEN MAGELLANICUS*.** J. Evan Ward\* and H. Keith Cassell, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF, Canada A1C 5S7.

The feeding activity of bivalve molluscs is a dynamic process; it is influenced by various physical and chemical factors in the environment, as well as the quantity and quality of particles in the surrounding media. Many previous workers have shown that changes in temperature, salinity, pH, particulate load, particle size, and perhaps particle shape can affect pumping, clearance, and ingestion rates. Few workers, however, have considered chemical signals as important factors in mediating bivalve feeding, even though chemically mediated feeding behavior in another class of molluscs, the Gastropoda, has been shown to be very important. In this study we demonstrate that the sea scallop, *Placopecten magellanicus*, increases its clearance and ingestion rates in response to metabolites from the diatom *Chaetoceros muelleri*. On average, clearance rates were 44% higher and ingestion rates 46% higher when scallops were exposed to soluble metabolites than when they were exposed to control media. Dose response curves indicate that the stimulation saturates at a low concentration of diatom extract (eq. to 5,000 cells ml<sup>-1</sup>). To our knowledge this is the first time this phenomenon has been demonstrated in a marine bivalve, and we suggest that chemical cues from microalgae are important factors which allow scallops to adjust feeding rates in nature.

**IN SEARCH OF THE IDEAL ALGAL DIET FOR OYSTERS: RECENT PROGRESS, WITH EMPHASIS ON STEROLS.** Gary H. Wikfors,\* National Marine Fisheries Service, Northeast Fisheries Center, Milford, CT 06460; Patricia K. Gladu and Glenn W. Patterson, University of Maryland, Department of Botany, College Park, MD 20742.

Differences in growth of bivalve mollusks on various algal diets are related to phytoplankton biochemical composition—especially lipids. Oysters, *Crassostrea virginica*, have a metabolic requirement for cholesterol but are unable to synthesize cholesterol from acetate, as do vertebrates. Most microalgae, including species that support rapid growth of oysters, contain very little cholesterol, *per se*, but do produce a number of other sterols, some of which are similar to cholesterol. A study now in progress is addressing the question of how various sterols present in microalgae affect the growth of oysters.

Thus far, sixteen algal strains have been evaluated for sterol composition semi-quantitatively, with component sterols measured as percentages of total sterol. Appreciable differences in

sterol composition were found between algal species. Under controlled laboratory conditions, for twelve weeks, young, post-set oysters were fed known rations of axenically-cultured algae; live weights of oysters were measured weekly. Two aspects of algal sterol structure, 1) the presence of an ethyl rather than a methyl group on carbon 24, and 2) the presence of a double bond at carbon 5 (cholesterol is in this group), showed statistically-significant positive effects upon oyster growth. A step-wise regression model ( $p = 0.025$ ,  $R^2 = 0.68$ ), using results from principal components analysis, identified these two sterol attributes—along with algal dry weight, protein, and carbohydrate rations—as accounting for 90% of the differences in oyster growth on the 16 algal diets.

## POSTER SESSIONS

**MORPHOLOGICAL AND GENETIC VARIATION OF *MYTILUS EDULIS* IN NEWFOUNDLAND.** J. Bates\* and D. J. Innes, Dept. of Biology, Memorial University, St. John's, Newfoundland, Canada, A1B 3X9.

Koehn et al. in 1984 conducted an electrophoretic survey of mussels from 5 sites along the Newfoundland coast and concluded that there were two species of blue mussels present which they called *Mytilus edulis* and *M. trossulus*. Work has been undertaken to confirm the presence of the two species and study their relative distributions. Eleven sites along the eastern coast of Newfoundland were sampled. Nine of these were represented by a single sample while two were sampled more extensively to examine microgeographic and size related trends. Four loci, Pgm Est Lap Ap, were used as these have proven useful in discriminating these two taxa in previous work. Two morphological criteria were also assessed; shell shape and inner shell colour. Shell shape has been previously shown to differ slightly between the two types. While shell colour has not been used before there is a fairly extensive body of literature indicating that shell colour in molluscs is likely to be genetically controlled. The results show that shell colour and enzyme genotype appear to be correlated, confirming the presence of two reproductively isolated taxa in Newfoundland. Furthermore the two taxa co-occur at almost all sites examined. However, morphological and genetic information suggest that the situation may be more complex than originally proposed.

**THE FATE OF HATCHERY-REARED JUVENILES OF *MYA ARENARIA* L. IN THE FIELD: HOW PREDATION AND COMPETITION ARE AFFECTED BY INITIAL CLAM SIZE AND STOCKING DENSITY.** Brian F. Beal,\* University of Maine at Machias, 5 O'Brien Avenue, Machias, ME 04654.

Since 1987, ten Downeast Maine coastal communities have participated in a unique shellfish management program based on stock enhancement using hatchery-reared softshell juveniles (8–12 mm SL). This non-traditional attempt to increase local pro-

ductivity has occurred at a time when Maine's clamming industry has experienced a 65% decline in landings.

During the third week of May 1990, three separate sizes of soft-shell clams (Small— $\bar{X}$  SL = 5.1 mm  $\pm$  0.07 SE, n = 217; Medium— $\bar{X}$  SL = 8.3 mm  $\pm$  0.06 SE, n = 184; Large— $\bar{X}$  SL = 11.7 mm  $\pm$  0.18 SE, n = 130) produced at the Beals Island Regional Shellfish Hatchery were added to 150 open field enclosures (15 cm diam  $\times$  15 cm deep) at each of two stocking densities (660/m<sup>2</sup> and 1320/m<sup>2</sup>) at each of three intertidal sites in eastern Maine. Two sites were located in the Chandler River, Jonesboro (a low intertidal area at the mouth of the river, and a high intertidal area 1 km above first site) while the third was a low intertidal area in Holmes Bay near the town of Cutler. During the first week of October, thirty enclosures, representing five replicates of each treatment combination, were randomly sampled from both low intertidal sites. Sixty enclosures (10 replicates/treatment) were sampled from the high intertidal site.

No differences in survivorship were detected between the two low intertidal sites ( $P = 0.34$ ). The effect of initial clam size on survival acted similarly between these sites ( $P = 0.46$ ) and was highly significant ( $P < .001$ ). Large clams had higher survival rates (70.0%  $\pm$  3.05 SE, n = 20) than medium-size clams (38.8%  $\pm$  6.35 SE, n = 20) ( $P < .001$ ) which survived better than small clams (15.3%  $\pm$  4.07 SE, n = 20) ( $P < .001$ ). The effect of stocking density on survival was unimportant at either site ( $P = 0.38$ ). Neither initial clam size ( $P = 0.70$ ) nor stocking density ( $P = 0.28$ ) affected clam survival at the high intertidal site where a rate of 43.5%  $\pm$  6.24 SE (n = 60) was observed.

Dead clams were found with either no visible shell damage, a countersunk hole drilled near the umbo (presumably from *Lunatia* spp.), chipped, or completely crushed (the latter two damage types attributable to crustacean predators). Approximately 5% of all clams recovered at the upper intertidal site had undamaged shells. This rate was significantly higher than the other two sites, and was unaffected by either clam size or stocking density. Mortality caused by boring gastropods was important only at the high intertidal site where 36.1% had been drilled. *Lunatia* spp. apparently exhibited no functional response ( $P = 0.96$ ) and was not attracted to a certain clam size ( $P = 0.82$ ). Crushed or chipped shells were more common at the two lower intertidal sites as 26.7% of all clams recovered fell into this damage category at Cutler compared with 11.3% at Jonesboro. At Cutler, crustacean-induced mortality was nearly three times greater on small and medium size clams than larger ones (33.9% vs. 11.9%;  $P = .03$ ). At Jonesboro, small clams were attacked more frequently (28.8%) than either large or medium clams (5.3%) by crustaceans ( $P < .005$ ). Initial density did not affect mortality rate at either low intertidal site. These results suggest that communities participating in public stock enhancement programs will attain the greatest return on their investment by transplanting the largest hatchery-reared individuals they can obtain.

**REPEATABILITY OF TRIPLOID INDUCTION IN *CRASSOSTREA VIRGINICA* (GMELIN) USING STRIPPED GAMETES.** David Bushek\* and Standish K. Allen, Jr., Haskin Shellfish Research Laboratory, Rutgers University, P.O. Box 687, Port Norris, NJ 08349.

Successful production of triploid oysters requires precise control of the timing of fertilization of eggs. Logistically, using stripped gametes is the most practical method for producing triploids because the time of fertilization can be easily controlled. We examined several factors that may affect the survival and the yield of triploid *C. virginica* from stripped gametes during large scale spawns.

Triploidy was induced by inhibiting polar body II with cytochalasin B. Variables examined included: time allowed for stripped eggs to ripen, average female fecundity, egg density during treatment, development rate, percent of eggs exhibiting first polar body extrusion at treatment initiation, and treatment order. Fourteen groups of 17 to 49 M eggs were treated. Survival and percent triploidy of 48 hour old larvae were not correlated: mean survival was 22% and mean triploidy was 79%. Survival to the eyed stage was about 6%. A sample of 138 spat was 72% triploid indicating that the triploid estimate from 48 hour larvae was accurate. Overall, 400.8 M eggs yielded about 17.8 M triploid spat, an efficiency of 4.4%. All linear regression models incorporating the measured variables were not significant. The variability observed in triploidy and survival appears to be random, dependent upon egg quality or both.

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**DISTRIBUTION AND RETENTION OF *VIBRIO VULNIFICUS* BY TISSUES OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*.** Gesa M. Capers\* and Mark L. Tamplin. U.S. FDA, Fishery Research Branch, Dauphin Island, Alabama and University of Florida, Gainesville, Florida.

*Vibrio vulnificus* is a human pathogen often associated with *Crassostrea virginica*. It was measured in individual tissues of freshly harvested oysters and in oysters subjected to depuration by ultraviolet light and filter-sterilized seawater. *Vibrio vulnificus* was enumerated with the most probable number technique in homogenates of whole oyster tissues and in individual tissues, including the hemolymph, digestive region, gills, mantle, and adductor muscle. *Vibrio vulnificus* was identified by enzyme immunoassay. In freshly harvested summer oysters, the digestive region contained the highest concentration of *V. vulnificus*, with decreasing numbers in the adductor muscle, mantle, gills, and hemolymph. Concentrations and distribution of *V. vulnificus* in oyster tissues differed for warmer and colder months. Depuration did not reduce *V. vulnificus* numbers in whole oysters, but changed their distribution in tissues. Specifically, *V. vulnificus* concentrations increased in the adductor muscle, mantle, and gills. It replicated in tissues of oysters and was released at a rate of

approximately  $10^5$  to  $10^6$  *V. vulnificus* per oyster per hour. These findings indicate that *V. vulnificus* is part of the microbial flora of oysters.

**EVALUATION OF A GENE PROBE ASSAY FOR THE DETECTION OF ENTERIC VIRUSES IN GREAT BAY SHELLFISH.** Karen J. Chaput\* and Aaron B. Margolin, Department of Microbiology, University of New Hampshire, Durham, NH 03824.

Due to inadequate sewage treatment systems, the shellfish growing waters of the Great Bay estuary of New Hampshire are becoming increasingly contaminated with fecal pollution. State regulatory agencies monitor fecal pollution in growing waters by the use of total and fecal coliform standards. Although these organisms readily detect bacterial contamination, the reliability of these procedures to demonstrate the existence of viral contamination is doubtful. Viral associated illness, including Hepatitis A, has occurred in individuals consuming shellfish from growing areas meeting all standards for harvesting. Current accepted methods to detect viral contamination, such as cell culture, can be time consuming and lack the sensitivity needed to detect low levels of enteric viruses found in shellfish. For example, hepatitis A virus (HAV) takes up to 3 months to replicate in cell culture, making monitoring by this method impractical. Gene probes are a rapid and sensitive method which has been developed to evaluate viral contamination by the detection of viral deoxyribonucleic acid (DNA) and a ribonucleic acid (RNA).

Hepatitis A virus, strain HM175, was used to seed shellfish eluents to evaluate the efficacy of a gene probe assay. Preliminary results demonstrate the effectiveness of this assay to detect enteric viruses in shellfish. To demonstrate the use of the assay with environmental samples, oysters were collected from the Great Bay estuary in areas opened and closed to shellfish harvesting. Samples were taken monthly from October, 1989 to September, 1990. Shellfish samples were evaluated for poliovirus by gene probes and cell culture. Samples were also evaluated for HAV by gene probes.

**EFFECT OF SALINITY OF *PERKINSUS MARINUS* SUSCEPTIBILITY AND DEFENSE-RELATED ACTIVITIES IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*.** Fu-Lin E Chu\* and Jerome F. La Peyre, Virginia Institute of Marine Science, the College of William and Mary, Gloucester Point, VA 23062.

Infection caused by *Perkinsus marinus* and defense-related activities in eastern oysters maintained under different salinity treatments were determined. Oysters were injected with trophozoites ( $10^6$  trophozoites per oyster) of *Perkinsus*, incubated in a closed system for six weeks in water of 3, 10 and 20 ppt. Oysters were then sacrificed and assessed for *Perkinsus* infection. Simultaneously, defense-related activities of individual oysters from each

treatment group including controls were examined. Prevalence and intensity of *Perkinsus* infection was found to be correlated with salinity; both factors increased with salinity. Heavy infections were found in both 10 and 20 ppt but not in 3 ppt groups. Total hemocyte count in oysters maintained at 10 ppt was significantly higher than at other salinities. Lysozyme concentrations were significantly lower in the oysters maintained in 10 and 20 ppt water than those maintained in 3 ppt water. There was a trend of increasing hemagglutinin levels in oysters with increasing salinity. At salinity higher than 3 ppt, hemagglutinins were lower in *Perkinsus* challenged than in control oysters.

**SELECTIVE BREEDING FOR INCREASED GROWTH RATE OF EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) IN A MAINE ESTUARY.** Christopher V. Davis,\* Robert O. Hawes, and Herbert, Hidu, Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469.

In response to a regional need for genetically improved lines of hatchery reared oyster seed, a ten year initiative began in 1986 to improve the cold water growth performance of eastern oysters through selective breeding regimes. Three regionally distinct parental lines of broodstock, (Flowers, Milford and Rutgers), were selected following eighteen months of growth trials under uniform environmental conditions. Parental "select" lines of broodstock were composed of individuals within the top 20% of the weight distribution based on whole wet weight. "Control" lines were composed of randomly chosen individuals from all weight classes of the original population. Spawning occurred in 1988 and offspring were reared for two growing seasons in floating screened trays in the Damariscotta River, Maine. In all three lines, offspring from selected parental stocks outgrew the controls when measured in terms of whole wet weight after 21 months growth. Whole wet weight differences of 6.8%, 13.1% and 56.6% were observed between the select and control groups of the Milford, Flowers and Rutgers lines respectively. All t-test differences were significant at the  $p < .10$  level or less.

**NATURAL SPAWNING VERSUS STRIP SPAWNING: A PRELIMINARY EVALUATION OF SUCCESS BETWEEN THE TWO METHODS IN *CRASSOSTREA VIRGINICA*.** Gregory A. Dehrosse\* and Standish K. Allen, Jr., Rutgers University, Haskin Shellfish Laboratory, Port Norris, NJ 08349.

In addition to naturally induced spawns, eggs and sperm may be obtained by dissection, or strip spawning. Successful natural spawns are dependent upon the oyster's willingness to spawn when subjected to thermal or chemical stimulation, or both. When the gametes are to be used in controlled genetic crosses, natural spawning can be problematic. Rarely does a significant portion of oysters spawn on cue; almost never will specific individuals cooperate; and cross contamination of gametes is always a possibility.

Conversely, stripping ripe oysters makes it possible to obtain the desired number of gametes from specific individuals on cue, without contamination. Do both methods result in comparable larval survival?

Hatchery culture records for 1988–1990 were used to evaluate percent survival of larvae over the first 48 hours and subsequently, up to the appearance of the first “eyed” larvae. Data from 14 natural and 12 strip spawns were included in the evaluation. Contrary to conventional dogma, we found that survival during the first 48 hours was higher (mean = 41.3%) in larvae from stripped eggs than from natural spawns (mean = 29.5%). However, the reverse was true when comparing survival to the eyed stage. Survival to first eyed was 27.2% in larvae from natural spawns, but 11.0% in larvae from stripped eggs. Most of the natural spawning took place during 1988–89, while strip spawning was the sole method used during 1990; it is unclear whether the lower survival of stripped cultures in this comparison is merely an artifact of between-season culture conditions. Further comparisons are planned during the 1991 season to resolve this question. NJAES Publication no. K-32100-2-91.

**MOVEMENT OF OFFSHORE LOBSTERS (*HOMARUS AMERICANUS*) DISPLACED TO COASTAL AREAS OF NOVA SCOTIA.** David R. Duggan, Benthic Fisheries and Aquaculture Division Biological Science Branch, Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S., Canada B3J 2S7.

A total of 593 berried female lobsters were displaced from Georges Bank to three coastal locations along Nova Scotia's southwestern shore. Of the total released, 106 (18%) were recaptured. Most (93%) recaptured lobsters had moved offshore in the direction of original capture and were caught >100 km from release sites. Mean days at large was 467 and the greatest time at liberty was 1526 days. The mean distance travelled was 148 km and mean change in depth was +163 m. The greatest straight-line distance was 264 km, recorded for a 128 mm lobster which travelled from Pubnico, N.S. to the Corsair Canyon area on Georges Bank in 350 days. Of the 106 recaptured lobsters, 12 (11%) were recaptured a second time. Results of this ongoing study indicate that displaced offshore lobsters exhibit a directed movement toward the area of original capture. Further tagging studies are required to gain more insight into movement patterns of mature coastal lobsters.

**SEASONAL VARIATION IN RESPONSE TO LABORATORY CONDITIONING IN *MERCENARIA MERCENARIA* IN SOUTH CAROLINA.** J. G. Goodsell\* and A. G. Eversole, Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson, SC 29634-0362.

Hard clams, *Mercenaria mercenaria*, (n = 300) were collected from a field population in August and November (Fall),

February (Spring) and May and June (Summer) in two consecutive years and conditioned in the laboratory. Temperature in the conditioning tank was maintained at 19°C ± 1°C, salinity varied 27–30 ppt, feeding rates were maintained at 1–2 X 10<sup>9</sup> cells/clam/day of *Isochrysis galbana* (T-ISO) and/or *Chaetoceros gracilis*. During each seasonal trial, attempts were made to induce spawning (N = 40) at 0, 3, 4, 6, 8, 10 and 12 weeks after starting conditioning. Spawning trials were rated on number of males and females spawning, number of gametes produced, egg size and larval survival to 48 hours. A random sample of clams (n = 10) from each trial were saved for histological analysis. A principal components analysis was used to create an index (Score = 0.480350% males spawning + 0.500924% females spawning + 0.513265 total % spawning + 0.260302 number of sperm produced + 0.112338 number of eggs produced – 0.340781 egg diameter – 0.241639% larval survival) for conditioning season and for week within conditioning season. “Best” spawns were ranked 6 and poorest were ranked 1. Weeks in which no spawn occurred were unranked. As expected, best conditioning season was summer followed by spring and fall. This order coincided with natural spawning pattern in South Carolina. In both years, clams collected in summer required little or no conditioning before an adequate number of clams spawned (>30%) were producing a large number of viable eggs (>10<sup>6</sup>) and sperm (10<sup>9</sup>). When fertilized, these gametes yielded survival rates to straight hinge stage >50%. Optimum conditioning time in summer was 10 weeks (Score = 6). During spring trials in year 1, males spawned on weeks 8 and 12, and females spawned on week 12 only. In year 2, spawning increased steadily through week 8, with 82% spawning, (Score = 5) then decreased (week 12, Score = 2). In fall year 1 (November), total number spawning never exceeded 10% and no females spawned. In fall year 2 (August), clams began to spawn at week 6 (Score = 3) and continued, increasing slightly through week 12 (Score = 4).

**PHAGOCYTOSIS OF *VIBRIO VULNIFICUS* BY *CRASSOSTREA VIRGINICA* HEMOCYTES.** Linda H. Hopkins,\* Mark L. Tamplin, and William S. Fisher, Jacksonville State Univ., Jacksonville, AL and Univ. of Alabama in Birmingham, Univ. of Florida, Gainesville, FL, and Univ. of Texas, Galveston, TX.

*Vibrio vulnificus* is a naturally occurring marine bacterium which can cause invasive human disease following consumption of raw oysters. This bacterium is believed to be natural flora of the Gulf Coast oyster, *Crassostrea virginica*. To better understand the relationship between *V. vulnificus* and oyster tissues, we examined the phagocytic response of oyster hemocytes to temperature, time, hemocyte:bacterial ratios, serum concentration, and translucent and opaque strains of *V. vulnificus*. The results showed that the number of hemocytes binding bacteria increased with time, ratio, and temperature. Greater than 60% of hemocytes bound

translucent strains, whereas only 20% were associated with opaque strains. This indicates that opaque strains of *V. vulnificus* may resist phagocytosis as previously described for mammalian phagocytes. Preincubation of bacteria in serum or increased concentration of serum did not affect the rate of phagocytosis, indicating a lack of opsonic effect. Understanding these cellular interactions may explain how *V. vulnificus* is retained in oyster tissues and its ecology in estuarine environments.

**QUANTITATIVE RELATIONSHIPS BETWEEN POST-LARVAL LOBSTERS AND NEW BENTHIC RECRUITS IN THE BOOTHBAY REGION OF MAINE.** Lewis S. Incze, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575 and Richard A. Wahle, Brown University, Providence, RI 02912.

Lobsters recruit to the benthos from a planktonic, primarily neustonic, postlarval (PL) stage. Along the coast of Maine, recruits are found primarily in shallow subtidal cobble (<10 m below MLW) which is patchily distributed and comprises a small portion of total bottom area. We measured densities (No. m<sup>-2</sup>) of new recruits at six sites in a coastal bay and at two coastal island sites in September 1989 and 1990 and compared these data to densities of neustonic postlarvae sampled from late July to late August of the same years. New recruits (carapace length ≤10 mm) were more than two orders of magnitude more concentrated than PLs. We develop a simple diagnostic model of the pelagic to benthic transition in lobster recruitment that accounts for this increase in density, and we use this model to suggest plausible rates of PL diving and settlement that describe average and site-specific recruitment in the two years. Advection criteria and implications for import and mortality rates of PLs are evaluated.

**THE EFFICACY OF BLUE COLORMORPHIC AMERICAN LOBSTERS IN DETERMINING THE FEASIBILITY OF HATCH AND RELEASE PROGRAMS.** C. J. Irvine,\* R. Bayer, and B. Beal, Department of Animal, Veterinary, and Aquatic Sciences, University of Maine, Orono, ME 04469, S. Chapman, Ira C. Darling Center, University of Maine, Walpole, ME 04573 and D. A. Stubbs, Department of Psychology, University of Maine, Orono, ME 04469.

The feasibility of lobster hatch and release programs is currently being evaluated by researchers at the University of Maine via the release of blue colormorphic American lobsters, *Homarus americanus*. Presently, the efficacy of using these blue lobsters as biological markers is unknown due to the possibility of inherent behavioral differences in comparison to normal American lobsters. The basic behaviors analyzed in this study are: swimming, burrowing, walking, and lack of activity. At the Ira C. Darling Center in Walpole, Maine blue and control (normal) post-larval American lobsters, ranging in carapace length (CPL) from 4.5

mm to 6.5 mm, were filmed individually with a camcorder. Each trial lasted for a minimum of 15 minutes and none exceeded 30 minutes. Six different substrates and three approximate carapace lengths of 4, 5, and 6 mm were used. The substrates included: mud, sand, eelgrass over mud, eelgrass over sand, pebble over sand, and cobble over sand. Five trials per substrate per carapace length were attempted for both blue and control animals. During the summer of 1990, a total 76 trials were conducted, 47 with blue lobsters and 29 with controls. The data was quantified by computing the relative time the lobsters spent in each of the behaviors in each trial. Of those trials analyzed to date no apparent behavior differences exist.

**ONTOGENETIC CHANGES IN SWIMMING ACTIVITY OF NORTHERN SHRIMP IN NON-FLOWING AND FLOWING WATER.** Mary-Jane James,\* Department of Zoology, University of Rhode Island, Kingston RI 02881.

Swimming behavior of laboratory reared northern pink shrimp, *Pandalus borealis*, zoeal stages I–V, was observed in a flume at several water flow velocities (0, 0.1, 0.2 and 0.4 cm/sec). Swimming speed and percent time swimming was measured, and swimming postures of the five zoeal stages were noted. Swimming speed increased in all water velocities for stage I to stage III zoeae. Stage III and IV were similar in their swimming speeds. In general, there was no difference in swimming speeds within zoeal stage at the different flow rates. Earlier stages, I and II, were weaker swimmers (approximately <0.05 cm/sec) than later stages, III and IV (approximately >0.10 cm/sec). There was no pattern in either flowing or non-flowing water in percent time swimming for zoeal stages I–IV. Zoeae in stages I and II swam in a vertical posture with the carapace below the telson. The pattern of movement was a slight bobbing in the vertical plane, with little or no displacement in the horizontal plane. On the other hand, swimming posture of later stage zoeae, III and IV, was inclined, the carapace below the telson. The direction of swimming was primarily forward in the horizontal plane. Stage V did not exhibit any swimming behavior and either sat or crawled on the bottom of the flume, often holding the telson high off the flume floor. This behavior suggests settlement occurs at this stage V.

Ontogenetic changes in swimming activity of *Pandalus borealis* zoeae may influence the distribution of larvae in the plankton, and therefore may be an important factor in the recruitment dynamics of this species.

**OYSTER GROW-OUT TECHNIQUES FOR THE MID-ATLANTIC: A DELAWARE BAY MODEL.** David R. Jones,\* Standish K. Allen Jr., and Stephen R. Fegley, Rutgers Oyster Research Laboratory, P.O. Box 687, Port Norris, NJ 08349.

A two-year project is currently underway to determine the economic feasibility of intertidal cultchless oyster production in Dela-

ware Bay. The first year of this project has just ended. Cultchless spat were produced from Rutgers University's MSX-resistant lines of *Crassostrea virginica*. Spat were reared in two upweller nursery systems located on the Atlantic and Delaware Bay coasts of New Jersey. When 8–10 mm in size, these spat were placed in plastic mesh cages and moved onto fiberglass racks at two intertidal sites in Delaware Bay. Spat reared on racks experienced 30–40% aerial exposure.

Of approximately 200,000 oysters placed intertidally, 75% survived the first growing season. There was considerable variation in growth and survival, with the fastest-growing oysters also showing highest survival. The Atlantic coast nursery showed slower growth than the Delaware Bay nursery. The middle Delaware Bay growout site showed slower growth than the lower Delaware Bay site. Harvesting of market-sized oysters is expected at the end of the second growing season in 1991. Detailed economic analysis will be used to show commercial feasibility of the techniques used. NJAES Publication Number K-32100-5-91.

**SALINITY AND TEMPERATURE TOLERANCE TESTS ON OCEAN QUAHOGS, *ARCTICA ISLANDICA* (L.).** M. Gayle Kraus and Brian F. Beal, Science/Math Div. Univ. of Maine at Machias, Machias, ME 04654, Louanne McMartin, Biology Dept., Univ. of Southern Colorado, Pueblo, CO 81001.

The ocean quahog occurs predominately in deep water ( $\leq 250$  m) from Newfoundland to Cape Hatteras. This distribution has led to a common assumption that these animals are intolerant of low salinities and high temperatures. Prior to the development of a culture program, we wished to determine the feasibility of using tidal flats as nursery sites.

The first phase of this investigation was to determine if juvenile quahogs ( $\bar{X} = 10.6$  mm SE  $\pm 0.10$ ) were able to survive low salinities for at least the length of a tidal cycle. Individuals used in this study were collected from commercial beds in Machias Bay (Lat. 40°35'N; Long. 67°26'W). Groups of ten juveniles were placed in each of six salinities (5, 10, 15, 20, 25 & 30‰) at two temperatures (6° & 19°C). Their behavior was recorded hourly for the first eight hours and periodically after that for the next 26 days. Death was defined as lack of response when the mantle was touched. Seawater was changed and quahogs were fed 5 ml of cultured algae (*T. Isochrysis galbana*,  $6-9 \times 10^6$  cells/ml) daily for the first 10 days and a minimum of five times a week thereafter. LD<sub>50</sub>'s at 19°C were 7 and 11 hr for 5‰ and 10‰, respectively. Two of the ten individuals in 10‰ lived (periodically siphoning) for 20 days. LD<sub>50</sub>'s for the same salinities at 6°C were 11 and 24 hr, respectively. Except for a single individual in four of the remaining eight treatments, all quahogs in salinities  $\geq 15$ ‰ at both temperatures survived for 27 days, when the experiment was terminated. These quahogs remained active, periodically siphoning, during this time.

**DESIGN OF A PC-COMPATIBLE BIOECONOMIC PROGRAM USED IN FORECASTING THE BARATARIA BAY OYSTER FISHERY.** Jimmy J. Landry,\* Department of Engineering Technology, Nicholls State University, Thibodaux, LA 70310; Earl J. Melancon, Jr., Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310.

The computer program, written in compiled BASIC, is based on a bioeconomic model developed for the southeast coast of Louisiana, a high salinity region.

Designed for use by individual oystermen or state agencies, the program predicts the number of sacks that can be harvested and the gross and variable production cost.

Program features include: Data file management, graphical display of results, user friendly menu driven, PC compatible (CGA, EGA mode), print report on dot matrix printer, and new models can be integrated with little effort.

**COLLECTING JUVENILE SEA SCALLOPS (*PLACOPLECTEN MAGELLANICUS*) WITH ARTIFICIAL COLLECTORS, IN PORT AU PORT BAY, NEWFOUNDLAND (CANADA).** Marc Lanteigne, L.-A. Davidson, and J. Andrews, Fisheries and Oceans, Gulf Fisheries Centre, Box 5030, Moncton, New-Brunswick, Canada E1C 9B6.

The supply of seed (juvenile scallops) for aquaculture activities is one of the major limiting factor for the advancement of the sea scallop aquaculture in Atlantic Canada. Due to the cost of hatchery produced seed, the industry has had to rely on collecting wild seed, using artificial collectors (Japanese onion bags). Collecting sea scallop seed from the wild has always been unpredictable, producing quantities of seed barely acceptable for commercial activities (approx. 300 juveniles/collector).

In 1988, the Port au Port Bay became well known as an excellent site for juvenile collection. With the improvement of the collecting techniques and the particularities of the site, aquaculturists were able to collect consistently more than 3000 juvenile scallops/collector from 1988 to 1990.

In 1988, the Department of Fisheries and Oceans initiated a project to study the recruitment mechanisms in Port au Port Bay. The results of three (3) years of collecting juvenile scallop are presented and discussed.

**DEVELOPMENT OF A NUCLEIC ACID PROBE FOR *HAPLOSPORIDIUM NELSONI* (MSX).** D. T. J. Littlewood and Susan E. Ford, Haskin Shellfish Lab., Rutgers University, P.O. Box 687, Port Norris NJ 08349; Dunne Fong, Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

In order to detect the oyster parasite *Haplosporidium nelsoni* (MSX), rapidly and sensitively, we are taking advantage of the

species specific structure of ribosomal nucleic acids to develop a molecular probe. Ribosomal nucleic acids are the most common in any eukaryotic cell. After denaturation of each strand of these double stranded molecules will reanneal specifically with their complement or may hybridize with other complementary single stranded molecules, such as a radioactively or non-radioactively labelled probe. We are pursuing a non-radioactive probe which will be used in the diagnosis of MSX and to complete details of the MSX life cycle.

DNA was isolated from oyster sperm, uninfected oyster blood and MSX infected blood samples. Small subunit ribosomal DNA (SSrDNA) was amplified in each sample using the polymerase chain reaction technique with primers that anneal to the termini of SSrDNA. SSrDNA was cloned into suitable plasmid vectors which, in turn, were transfected into competent host *E. coli* cells. Purified recombinant plasmid DNA was sequenced using the dideoxy chain termination reaction. Complete sequences of oyster and putative MSX rDNA indicate these genes to be 1799 and 1793 bp respectively. Computer aided analysis of these genes will enable us to synthesize sequences (<30 bp) that are species specific. Preliminary probes will enable us to confirm that we have sequenced MSX, by *in situ* hybridization, before the probe and its use are perfected. This is NJAES publication number K32901-1-91.

**SCANNING ELECTRON MICROSCOPY (SEM) OF MUGARDIA, FORMERLY ANOPHRYS, A PATHOGENIC PROTOZOAN OF THE AMERICAN LOBSTER. M. B. Loughlin\* and R. C. Bayer.** Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469.

During spring, 1990, several lobster pounds experienced high mortalities caused by a ciliated protozoan. This study examined infected lobsters from tidal lobster pounds in Maine. Previous literature referred to this organism as *Anophrys*, recently the Society of Protozoologists reclassified this protozoan in the class Oligohymenophorea, subclass Hymenostomatia, order Scuticociliatida, family Paranophryidae, genus *Mugardia*. *Mugardia* is apparently an opportunistic marine species, free living but infecting various decapods through damaged and possibly diseased areas of the shell. *Mugardia* infects nearly all tissue causing death in three days to three weeks.

Live lobsters examined in this study showed weakness and lethargy. Light microscopy of infected hemolymph revealed a highly motile ciliate. *Mugardia* from an infected Maine pound were collected, preserved with buffered formalin fixative, prepared for SEM by ethanol/amyl acetate dehydration, CO<sub>2</sub> critical point drying and sputter coated with gold to 30 nm. SEM and VHS Video revealed; *Mugardia* cilia attached to lobster hemocytes, oral groove, kinetid and dikinetid structure and arrangement.

**RELEASE OF LARGE NUMBERS OF SMALL SYMBIOTIC CELLS BY THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS* IN NEWFOUNDLAND. Bruce A. MacDonald, J. Evan Ward, and Cynthia H. McKenzie,** Marine Sciences Research Laboratory, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's N.F., Canada A1C 5S7.

During routine feeding experiments in the field large numbers of symbiotic cells were released into suspension from within the mantle cavity of adult *Placopecten magellanicus*. The individual cells range in size from 6–12 µm and to date they have only been observed during summer months. Cells were recorded for approximately 10–20% of the individuals monitored in late June 1989 while up to 100% of the individuals in one mid-August 1990 sample (n = 9, males and females) contained at least some of the cells. Light microscopy and SEM were used to examine the external features of preserved cells which have not yet been identified.

**EFFECTS OF TWO SPECIES OF DINOFLAGELLATES ON THE NEW ZEALAND MUSSEL *PERNA CANALICULUS*. Islay D. Marsden,** Zoology Department, University of Canterbury, Christchurch, New Zealand; **Sandra E. Shumway,** Maine Department of Marine Resources, West Boothbay Harbor, ME 04575 U.S.A.

New Zealand mussels were maintained in quarantine conditions with low food availability. Opening behaviour and byssus production over 24 h were measured at approximately 5 day intervals over a 30 day experimental period. At 20°C mean byssus production ranged from 3.43 to 6.17 per day. Byssus production decreased with time corresponding to changes in the dry weight condition index. Individual *P. canaliculus* were exposed to 10<sup>5</sup> cells/L red tide of *Protogonyaulax tamarensis* (GT429) and (PLY173). There was no significant change in byssus production or opening behaviour between experimental groups and mussels produced normal faeces within 24 h. Oxygen uptake of individual *P. canaliculus* (dry weight 54 to 127 g) was measured prior to exposure to (GT429) and (PLY173), after 1 h exposure and following a 24 h recovery period. Oxygen uptake was similar for all conditions, and time intervals. It is concluded that although the New Zealand mussel actively feeds on toxic algae there is no significant short term effect on its oxygen uptake. Like other mussels however, continued exposure to GT429 results in the accumulation of toxins to levels unsuitable for human consumption.

**GROWTH, DISEASE RESISTANCE AND SURVIVORSHIP OF DIPLOID AND TRIPLOID EASTERN OYSTERS IN AN MSX ENVIRONMENT. George C. Matthiessen,** Ocean Pond Corporation, Fishers Island, NY 06390; **Jonathan P. Davis,\*** Baywater, Inc., Bainbridge Island, WA 98110.

The purpose of this study was to evaluate the potential for using triploid eastern oysters in a commercial oyster grow-out site

having a history of MSX. Triploid oysters were produced in the hatchery using standard induction methodologies in 1988 on Fishers Island, NY, and again in 1990 in Dennis, MA. Parents (1 male and 1 female) used in 1990 were themselves individuals selected for seven generations for resistance to MSX (parent stocks were obtained originally from Rutgers University Research Laboratory, strain designation R-BLA), while 1988 parental stocks (4 females and 7 males) were Ocean Pond (Fishers Island, NY) non-resistant oysters. In both years, individual triploid and diploid control oysters were placed for grow-out in Cotuit, MA, a commercial site having a history of MSX infestations.

Growth rates of triploid oysters exceeded that of diploids in both years, although the triploid yield in the 1988 group was very low (3%); nonetheless triploid oysters were significantly larger than their diploid siblings at fifteen months post set. The same result was observed in the 1990 group at six months post set; triploid oysters were significantly larger than their diploid controls. The controls in this experiment were diploids originating from the same broodstock population of seventh generation resistant oysters.

The pattern of disease resistance and mortality in Cotuit Bay diploid and triploid oysters was examined in August, September, and October, 1990. Though incidence of infection was higher in triploids throughout this period, mortalities attributed to MSX were significantly higher in diploids. These results are discussed within the context of utilizing triploid oysters for enhanced growth and survivorship potential in commercially productive MSX environments.

**A BETTER USE OF STOCK-SITE COMBINATIONS TO INCREASE BLUE MUSSEL PRODUCTION IN THE MAGDALEN ISLANDS (QUEBEC, CANADA).** Bruno Myrand and Jean Gaudreault,\* Direction de la recherche scientifique et technique, MAPAQ, C.P. 658, Cap-aux-Meules, Québec, Canada, G0B 1B0.

Mussel (*Mytilus edulis*) spat collected from 4 sites (=stocks) were transferred to 5 different growing site in November 1989 in order to create 20 different stock-site combinations. All combinations were then followed monthly from June to November 1990. Length-flesh DW and length-shell weight regressions were established for each combination. We estimated the commercial production per cage using these regressions applied to the length distributions (mussels  $\geq 50$  mm). The condition index (flesh DW/shell weight) was also calculated.

Mussels from the Amherst Lagoon (BHA) stock had a survival rate of 92.8% whereas the stock from Great Entry Lagoon (GE) exhibited a poor 22.8%. The survival rate had a strong impact on the estimated commercial production per cage as the mean length (47 to 51 mm) and the mean condition index (17.7 to 20.1%) of the mussels in November showed little variation. The estimated commercial production was 9.8 times higher for the BHA stock

(170.7 g/cage) than for GE stock (17.4 g/cage). The best site was the House Harbour Lagoon with 105.7 g/cage and the worst was the Great Entry Lagoon with 66.8 g/cage. The most interesting fact is that the mussel industry in the Magdalen Islands is located in Great Entry Lagoon and uses the local GE seed. In 1990, the commercial production could have been multiplied by an order of magnitude if mussel growers had merely used BHA mussels instead of the local ones.

**GROWTH PATTERNS OF FEMALE OFFSHORE LOBSTERS.** Douglas S. Pezzack,\* Dept. Fisheries and Oceans, Scotia-Fundy Region, P.O. Box 550, Halifax, N.S., Canada, B3J 2S7.

Offshore lobsters (*Homarus americanus*) inhabit the deep-water basins of the Gulf of Maine, the offshore Banks of Georges and Browns and the outer Scotian Shelf. Tagging studies conducted 1983–1988 tagged over 16,000 female lobsters, most of which were mature and over half were egg bearing at the time of tagging. Over 30% of the tagged lobsters were recaptured and 16% of these more than once. Growth increment per molt and the proportion molting were determined for egg bearing and nonegg bearing females. Though the standard pattern of alternating molting and egg extrusion was observed in the majority of cases, some mature females were observed to extrude eggs two successive years without an intervening molt; molt two successive years without an intervening egg extrusion; and molt and extrude eggs in the same season. These patterns may be the result of the warm year round temperatures offshore and unique to that area or they may represent the normal variability in growth strategies, not observed in other tagging studies due to lower return rates and the lack of large numbers of mature females.

**EGG PRODUCTION OF LOBSTER (*HOMARUS AMERICANUS*) IN THE GULF OF ST. LAWRENCE, CANADA.** Fernand Savoie\* and Donald R. Maynard, Department of Fisheries and Oceans, Invertebrates Division, Science Branch, Gulf Region, P.O. Box 5030, Moncton, N.B., E1C 9B6.

The female population of *Homarus americanus* in the Gulf of St. Lawrence was studied to determine the geographic and seasonal variations in fecundity. A total of two hundred berried female lobsters ranging from 60 to 120 mm carapace length were collected by trap during the fall and again in the spring at five sample sites within the Gulf. The number of eggs on each female was estimated and a regression of carapace length versus fecundity plotted for each sample site per season. Samples collected in the fall consisted of lobsters carrying newly extruded eggs while spring samples coincide with lobsters carrying eggs which will hatch within six to eight weeks. Percent egg loss between the two seasons varies from 15 to 27%. Geographic variation of fecundity and egg loss is discussed.

**OVERWINTERING SURVIVAL OF TRIPLOID EASTERN OYSTERS.** G. M. Shatkin,\* Department of Animal, Veterinary, and Aquatic Sciences, University of Maine, Orono, ME 04469 and Standish K. Allen, Jr., Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

We are presently engaged in experimental grow out of triploid American oysters, *Crassostrea virginica*, in Maine in order to assess commercial potential. In the Damariscotta River, oysters are frequently grown in floating trays as juveniles. To avoid freezing damage during winter, oysters are stored in one of several ways: at high salinity oceanic sites, on bottom, or in cold humid air. Triploid and diploid control oysters were overwintered at a high salinity site at the Ira C. Darling Center, Walpole, Maine and underground in cold, damp storage at Mook Sea Farm, Damariscotta, Maine. As a control, replicates were held in a recirculating seawater system at a constant temperature of 12°C. The oysters ranged from 3–10 mm and were divided into 9 groups of 800 triploids and 9 groups of 800 diploids. Three replicates of each genetic type were overwintered at each of the three locations. Changes in total weight and percent moisture were calculated in relation to method of storage. Shell length and mortality were determined at the termination of winter storage and one month later. These measurements allowed us to determine relative survival and recovery from winter storage in triploids and diploids.

**FLOW CYTOMETRIC ANALYSIS OF PURE-CULTURES AND NATURAL PARTICLE ASSEMBLAGES—GOING FROM THE SUBLIME TO THE RIDICULOUS?** Sandra E. Shumway<sup>1,2</sup> and T. L. Cucci,<sup>2</sup> <sup>1</sup>Department of Marine Resources and <sup>2</sup>Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575 USA.

The introduction of flow cytometric techniques to marine science has enabled researchers to study feeding habits of various marine organisms. These studies were previously hampered by the lack of techniques to distinguish quantitatively between different particles of the same size. Flow cytometric techniques were first applied to feeding studies in marine organisms in 1985 using bivalve molluscs. These studies utilized pure cultures fed to animals in combination and we were able to estimate not only the clearance rate of individual cell types, but also their proportional occurrence in the pseudofaeces and faeces. More recently we have translated our laboratory studies into field studies and have focused on natural seston as a food source for filter-feeding organisms. Particles are analyzed for their fluorescing intensities as well as particle size (measured as volume and/or forward angle light scatter) and we have been able to monitor feeding rates as functions of both particle size and organic content. Data are presented for various animals fed on pure cultures as well as natural assemblages of particles.

**PATTERNS OF DISTRIBUTION AND ABUNDANCE OF LOBSTERS IN THE GULF OF MAINE: IDEAS ON THE CARRYING CAPACITY OF THEIR ENVIRONMENT.** R. S. Steneck, Oceanography Department, University of Maine, Orono, ME; R. A. Wahle, Program in Ecology and Evolutionary Biology, Box G-W, Brown University, Providence, RI; L. S. Incze, Bigelow Laboratory for Ocean Science, West Boothbay Harbor, ME; D. F. Belknap, Geology Department, University of Maine, Orono, ME.

Quantitative demographic censuses of the lobster *Homarus americanus* were conducted throughout the Gulf of Maine. Lobster population densities and body size correspond to shelter availability which is controlled by the regional geology. Local carrying capacity of lobster habitats changes ontogenetically in relation to lobster size/shelter size scaling. Early Benthic Phase lobsters (7–40 mm CL) are virtually restricted to cobble habitats, whereas Adolescent Phase lobsters (40–90 mm CL) are most abundant in boulder fields. Reproductive Phase lobsters (>90 mm CL) are rare in all shallow coastal habitats. Lobster landings (as mass per length coastline) over the past two decades suggest that within the Gulf of Maine the central Gulf has the highest carrying capacity for lobsters. Population densities decline sharply at outer coastal sites north of the Mt. Desert region of the Gulf of Maine. This decline corresponds to the virtual absence of Early Benthic Phase lobsters in cobbles despite our finding postlarvae in overlying water at each area. We hypothesize that lower population densities to the north result from cold water inhibition of lobster settlement. The decline to the south may result from the increasing rarity of suitable recruitment substrata.

**THE ROLE OF EELGRASS (*ZOSTERA MARINA*) IN THE RECRUITMENT OF THE BLUE MUSSEL (*MYTILUS EDULIS*) IN MAINE.** Frederick Short, Dept. of Natural Resources and Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH 03824; Herbert Hidu, Dept. of Animal, Veterinary and Aquatic Science, University of Maine, Orono, ME 04469; Heidi Hoven, Dept. of Plant Biology, University of New Hampshire; Linda Kindblum, Dept. of Oceanography, University of Maine, Walpole, ME 04573; Carter Newell, Great Eastern Mussel Farms, Tenants Harbor, ME 04860; Karen Verny, Dept. of Zoology, University of New Hampshire; Authur Mathieson, Dept. of Plant Biology and Jackson Estuarine Laboratory, University of New Hampshire.

Jordon Cove, an estuarine system from Jordan River to Mt. Desert Narrows on the central Maine coast, is a significant source of seed mussel (*Mytilus edulis*) for the "on-bottom" mussel cultivation fishery. Preliminary studies have suggested that mussel larvae and pelagic drifting juveniles are differentially transported upstream towards the widened embayment of Jordan Cove; further they suggest that primary larval settlement and subsequent juve-

nile movement occurs on eelgrass meadows within this confluence.

Our current studies are attempting to quantify the dynamics of larval and juvenile mussels associations within one of the extensive eelgrass meadows in Jordan Cove. Larval abundance shows substantial spacial variability, being consistently more abundant on flood than ebb tides. As of now, we have not shown larval depletion from water mussels crossing these eelgrass meadows. However, there is a high recruitment of larval mussels to eelgrass shoots. As many as 32,000 juvenile mussels/0.1 m<sup>2</sup> of eelgrass were found although mussel settlement is variable throughout the entire bed. Predation of juvenile mussels on eelgrass appears to be slight and the presence of juvenile seastars (*Asterias vulgaris*) does not occur before the mussels begin to leave the eelgrass bed. The stimulus for juvenile mussel departure from eelgrass blades may be triggered by the appearance of *A. vulgaris* or by the response of juveniles to chemical queue(s) from adult populations inshore of the eelgrass.

Through modelling, spatial and temporal patterns of larval and juvenile movement is being evaluated relative to eelgrass beds in order to determine sources of mussel seed. We have pin-pointed the timing and location of mussel spat efflux from eelgrass beds and potential secondary settlement areas for seed collection. Experimental placement of live and shell mussel cultch resulted in the capture of 1,000 to 20,000 mussels/m<sup>2</sup>. Recruitment rates were high in August, followed by emigration in September and October. Higher cultch densities of 15 to 20 liters per square meter appear to be commercially feasible for mussel seed procurement during years of high larval densities near eelgrass meadows and could enhance and improve blue mussel production.

**SEASONAL MOVEMENTS OF LOBSTERS IN THE GREAY BAY ESTUARY.** W. H. Watson, III and W. H. Howell, Zoology Department, University of New Hampshire, Durham, NH 03824.

The Great Bay Estuary in New Hampshire is an atypical habitat for lobsters in that it exhibits large temporal fluctuations in temperature and salinity. In a typical late winter or early spring, bottom salinity in the upper estuary often approaches lethal levels, and during the summer bottom temperature in Great Bay is at least 10°C warmer than at the coast. We have been testing the hypothesis that lobsters use behavioral mechanisms, such as seasonal migrations up and down the estuary, to avoid low salinity conditions in the late winter and spring, and to take advantage of warmer waters during the remainder of the year.

For the past 3 years we have been using both tag/recapture techniques and acoustic telemetry to measure the movements of lobsters throughout the Great Bay Estuary system. Results reported here are based on more than 10,000 animals we tagged

from January 1988 to December 1990, and 20 animals that were equipped with sonar transmitters and tracked for periods ranging from 2 weeks to more than 1 year.

In general, animals move into the estuary in the late spring and early summer, remain relatively stationary or move down the estuary during the summer, and then move further down the estuary in the fall to their overwintering sites. While most lobsters moved short distances, some traveled up to 40 miles. There is a year-round population of lobsters in the estuary, and seasonal shifts in its distribution are correlated with long-term fluctuations in temperature and salinity. There also appears to be some exchange between estuarine and coastal populations. This research was supported in part by a grant from NOAA (Sea Grant).

## GENETICS/POLYPLOIDY

**HYBRIDIZATION AMONG THREE SPECIES OF CRASSOSTREA.** Standish K. Allen, Jr., Rutgers University, Haskin Shellfish Research Laboratory, Port Norris, NJ, 08349 and Patrick M. Gaffney, University of Delaware, College of Marine Studies, Lewes, DE 19958.

Our research has focussed on clarifying the ambiguities of hybridization in the genus *Crassostrea*. Reciprocal crosses of *C. virginica* with *C. rivularis* and *C. gigas* were produced using single females as replicates crossed with pooled sperm from two males. Zygotes produced from these crosses were examined by epifluorescent microscopy during meiosis and early mitosis to confirm syngamy. Straight hinge (48 hr) larvae were analyzed by starch gel electrophoresis and flow cytometry to confirm formation of hybrid embryos. Growth and survival were monitored throughout larval life. Cytogenetic and electrophoretic analysis revealed the formation of hybrids zygotes and larvae, but larval survival was limited to about 10 days. Larvae stopped growing at about day 4, reaching a maximum length of about 80 μm. Studies on larval feeding using fluorescent beads indicated that growth limitation apparently was not caused by an inability to feed. Induced triploidy did not rescue hybrid failure. In only one replicate were metamorphosed spat obtained; electrophoretic analysis revealed that these were contaminants—not progeny derived from the parents.

In contrast, hybrids between *C. rivularis* and *C. gigas* were successfully produced. Fertilization rate and larval survival to 48 hrs were lower than parental crosses; subsequent larval survival of hybrids was intermediate to that of parental crosses; larval growth rate was about the same for all crosses, except *rivularis* (female) × *gigas* (male) which was slower. Electrophoretic and flow cytometric analysis confirmed hybridization. NJAES publication no. K-32100-1-91.

**ELECTROPHORETIC COMPARISONS OF DEEP-SEA, COLD-WATER SEEP MUSSELS IN THE GULF OF MEXICO.** Clark, Craddock,\* Robert C. Vrijenhoek, and Richard A. Lutz, Center for Theoretical and Applied Genetics and Department of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08903-0231.

Mussels sampled from a cold-water methane/sulfide seep at the base of the West Florida Escarpment and two cold-water hydrocarbon seep sites in Alaminos Canyon, east of Galveston, Texas, were compared electrophoretically at 24 loci. At the West Florida Escarpment site (26°02.4'N, 84°54.0'W, 3314 m depth), the population is composed of a single species with the exception of one individual which exhibited fixed differences at 6 of 24 presumptive gene loci. Electrophoretic analysis of mussels from the Alaminos Canyon sites (26°21.1'N, 94°30.3'W, 2340 m depth and 26°21.3'N, 94°29.7'W, 2222m depth) revealed the presence of at least two species which differed from each other at 16 of 24 loci. Neither of the species encountered in Alaminos Canyon is found at the West Florida Escarpment seep. The more common of the Alaminos Canyon species differed from the West Florida Escarpment species at 10 of the 24 loci, and the less common differed at 17 of the 24 loci. For comparative purposes, we examined specimens of the mytilid *Bathymodiolus thermophilus* from the Mussel Bed hydrothermal vent site along the Galapagos Rift (0°47.894'N, 86°09.210'W, 2486 m depth). To date these analyses have revealed distinctly different allozyme banding patterns for at least 12 of 15 loci from any of the mytilid species found at the Gulf of Mexico cold-water seeps. The allozyme data obtained permits use of Nei's genetic distance and UPGMA to construct a species dendrogram to further our understanding of taxonomic relationships among mytilids at a variety of deep-sea hydrothermal vent and cold-water seep environments.

**REPRODUCTIVE ISOLATION BETWEEN NATURAL POPULATIONS OF THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA* AND SOUTHERN QUAHOG, *M. CAMPECHIENSIS*, IN SOUTH CAROLINA.** Robert T. Dillon, Jr., Department of Biology, College of Charleston, Charleston, SC 29424.

The Indian River of Florida has recently been designated a "hybrid zone" between the two species of hard clams. But strictly speaking, a region cannot be considered a hybrid zone until more complete reproductive isolation is demonstrated between two species when the co-occur elsewhere. Here I report the occurrence of small numbers of *M. campechiensis* in large *M. mercenaria* beds near Charleston, SC. A large sample of about 10,000 clams contained 27 apparently pure *M. campechiensis* and 6 hybrids, judging from shell morphology and isozyme frequencies at four diagnostic loci. So although reproductive isolation between the two species is not complete, it is indeed much greater in South Carolina than in the Indian River.

**PHYSIOLOGICAL DIFFERENTIATION IN EASTERN OYSTERS.** Dawn E. Dittman\* and Susan E. Ford, Haskin Shellfish Research Laboratory, Rutgers University, P.O. Box 687, Port Norris, NJ 08349.

In species with large geographic ranges, local populations often show physiological differentiation correlated with temperature clines. Organisms living in colder temperature regimes compensate by increasing their metabolic rate at a given low temperature relative to those organisms living in warmer latitudes. This type of adaptation maximizes the energy available for growth and reproduction in a given thermal regime.

Strains of *C. virginica* from different thermal regimes (Long Island and Delaware Bay) have been maintained together in Delaware Bay for multiple generations. The situation is ideal for the separation of genetically based physiological variation from environmentally influenced differences (acclimatization). If genetically based physiological compensation has occurred, the population from farther north will show faster physiological rate functions at low temperatures, even after several generations.

We have analyzed growth data collected between 1977 and 1990. After up to seven generations in Delaware Bay, strains of Long Island origin grow faster and are significantly larger at a given age than oysters of Delaware Bay origin. An experiment on 1988 year class oysters showed that at low temperatures (-1°, 2°, & 6°C) the cilia of the Long Island strain were significantly ( $p < 0.05$ ) more active than the cilia of the Delaware Bay strain. At the lowest temperature (-1°) the Long Island oyster cilia were 48% more active than the Delaware Bay oyster cilia. This result is consistent with the hypothesis that faster growth of Long Island strains may be associated with remaining active at lower temperatures than the Delaware Bay strains. Further experiments on the variation of feeding, assimilation, and other physiological rate functions with temperature will explore geographic differentiation in these oyster populations. NJAES Publication No. K-32405-2-91.

**HYBRIDIZATION, TRIPLOIDY AND SALINITY EFFECTS ON CROSSES WITH *CRASSOSTREA GIGAS* AND *CRASSOSTREA VIRGINICA*.** Sandra L. Downing,\* School of Fisheries, WH-10, University of Washington, Seattle, WA 98195.

With pollution and disease decimating the populations of *Crassostrea virginica*, a hybrid with the hardier *Crassostrea gigas* could be one answer for revitalizing the east coast industry. During the spring of 1988, a complete factorial design was used to produce monospecific and interspecific, diploid and triploid oysters. This design will be repeated during 1991. To induce triploidy, newly fertilized eggs were treated with cytochalasin B from 20–35 min after insemination at 25 C. Salinity (18 to 30 ppt) was tested to determine its effects on triploid induction and rearing in general.

During the 1988 trials, survival to 48 hours was higher in the untreated diploid crosses (69%) than in the monospecific treated groups (46%). Although below control levels, *C. gigas* sperm readily fertilized *C. virginica* eggs, and in fact, both VG and VVG yielded good sets. However, in the reciprocal cross, fertilization was less successful: survival to 48 hours was <5% in GV and <1% in GGV crosses. Such low survival rates could possibly indicate spontaneous gynogenetic development or mean that any survivors might be contaminants. Therefore when the crosses are repeated this year, electrophoresis or PCR techniques will be used to confirm hybrid status of survivors. Similar to published results, there was a large die off around 7 days for all groups with *C. virginica* as the maternal parent.

When broodstock were conditioned at 28 or 30 ppt, larvae did not survive when treated at 18 ppt, but did between 20 and 30 ppt. Survival of triploid *C. virginica* groups was constant over the salinity range while survival decreased directly with salinity for *C. gigas*. During 1988, 100% triploid was induced in the 20VVV group while only 84% in the 30 ppt group. When this was repeated using *C. gigas* during 1989, 100% triploid was induced in all three salinity groups.

**HYBRIDIZATION IN *CRASSOSTREA*: A CRITICAL REVIEW.** Patrick M. Gaffney,\* College of Marine Studies, Lewes, DE 19958; Standish K. Allen, Jr., Rutgers University, Shellfish Research Laboratory, Port Norris, NJ 08349.

Numerous attempts at hybridization in the genus *Crassostrea* have been reported. Most suffer from one or more of the following: 1) ambiguities in the classification of oysters; 2) contamination of experimental cultures by extraneous gametes or larvae; 3) the presence or absence of control experiments for assessing the quality of gametes as well as larval viabilities; 4) the absence of genetic confirmation of hybrid status. We conclude that there is virtually no unequivocal evidence for the formation of viable interspecific hybrids among *Crassostrea* species.

Examples of taxonomic ambiguities that cloud interpretation of hybridization experiments include the frequent misclassification of the Kumamoto oyster (*C. sikamea*) as the Pacific oyster (*C. gigas*), the probably unjustified distinction between *C. gigas* and the Portuguese oyster *C. angulata*, and the lumping of *C. iredalei* with *C. rivularis*. Low-level contamination of cultures is a common occurrence that may account for many reports of successful hybridization. Experimental controls are essential to properly assess gamete quality and zygote growth and survival, yet are often not included. Finally, the majority of reports provide no genetic confirmation that the zygotes produced are actually hybrids. To date, most genetic analyses have demonstrated that putative interspecific hybrid oysters were not hybrids.

Use of adequate controls and genetic analysis of parents and offspring are essential to hybridization experiments, and are dem-

onstrated in an accompanying paper (Allen and Gaffney, this symposium).

**DETERMINATION OF "BEST PARENTS" IN A SPAWNING OF HARD CLAMS BY COMPARISON OF PARENTAL AND PROGENY GENOTYPES AT SIX ENZYME LOCI.** Nancy H. Hadley,\* Marine Resources Research Institute, Charleston, SC; Robert T. Dillon, Jr., College of Charleston, Charleston, SC.

As part of a selective breeding program for hard clams, genotypes of spawners and two year old progeny were compared to determine the best parents. The spawners were the largest 10% of a population of two year old siblings. 150 clams were stimulated to spawn in each of two experiments. In one experiment, 11 males and 12 females spawned. In the other, 38 males and 21 females spawned. Each spawner was marked with a unique code so they could be distinguished later. At two years of age, progeny of each experiment were segregated by size and approximately sixty each of the largest and smallest were sacrificed for electrophoretic determination of genotypes at 6 enzyme loci. Parental genotypes were determined, using a non-destructive sampling technique. A computer program was developed to determine the probability of each offspring resulting from each possible parental combination. Of the original 82 spawners, 16 died prior to sampling (6 females and 10 males). Of the remaining 66 spawners, one had no identifiable progeny among the two year old offspring. There were no parental possibilities for 60 of the 207 progeny sampled, indicating that one or both of their parents were among spawners which had died. Mothers were positively identified for 22 individuals, fathers for 33 and both parents for 13. Ten of the 13 offspring whose parents were positively identified were in the small group. Thirteen spawners were considered undesirable parents, having a greater probability of producing small offspring than of producing large. The remaining 52 (30 males, 22 females) were ranked according to their overall probability of producing large offspring. A respawning of these will be used to determine the utility of this technique for producing rapidly growing hard clams.

**STUDIES OF EMBRYONIC AND LARVAL RESPONSE TO SELECTION FOR INCREASED RATE OF GROWTH IN ADULT BAY SCALLOPS, *ARGOPECTEN IRRADIANS CONCENTRICUS*.** Peter B. Heffernan,\* Randal L. Walker, and John W. Crenshaw, Jr., Shellfish Research Laboratory, Marine Extension Service, University of Georgia, Savannah, GA 31416-0687.

F<sub>1</sub> brood stocks of *Argopecten irradians concentricus* were examined for the effect of truncation selection for increased growth rate in adults on the growth and survival rates of embryonic and larval stages. Embryonic viability levels were significantly lower (41–59%) in all (N = 3) selected groups tested when compared to the control (unselected) cohort. Significantly higher shell length

values were detected after 48 hours, in those groups which displayed the highest embryonic survival rates.

Larval progeny of the unselected brood stock had significantly higher shell length values at 10 and 14 days when compared with those from two selected brood stocks. Progeny of the unselected line also metamorphosed/settled earlier than those of selected lines. Larval phase survival levels appeared to be independent of the selection pressure imposed on their parents. These results support similar findings reported by this group highlighting the negative larval response to selection for increased growth rate in *Mercentaria mercenaria*.

**ASSOCIATION OF ALLOZYME HETEROZYGOSITY WITH LARVAL AND JUVENILE VIGOR IN AN EXTENSIVELY SELECTED OYSTER (*CRASSOSTREA VIRGINICA*) STRAIN.** Yaping Hu,\* Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08903.

To investigate the correlation between heterozygosity of allozyme loci and fitness related traits, such as survival and growth in the early development stages for a selectively bred (sixth generation) oyster strain from Delaware Bay, U.S.A., a standard mass spawning was conducted. Individual eyed larvae (shell length, approximately 300  $\mu\text{m}$ ) and juveniles (shell length, 3 to 15 mm) were analyzed electrophoretically for the phosphoglucose isomerase (Pgi) locus. The first larvae reaching metamorphosis exhibited a higher percentage of heterozygous individuals (88%) than the last larvae (7 days later) reaching metamorphosis (58%). In the subsequent juvenile stages, 65% of individuals in the fast growing group were heterozygotes, whereas 54% of the slow growing group were heterozygotes. The observed heterozygosity in both early metamorphosing larvae and fast growing juveniles was significantly higher than Hardy-Weinberg expectations (approximately 50%).

Consistent results also occurred in juvenile oysters from pair matings of known genotypes. Heterozygosity ranged from 51 to 73% (average 64%), significantly in excess of the expected Mendelian ratio (50%). The mechanism by which heterozygosity marked by Pgi affects the fitness traits remain unclear. The Pgi locus itself may affect development and growth, or the entire chromosomal region may be involved in such control. Nevertheless, this apparent heterosis might serve as a useful genetic indicator for oyster breeding and hatchery programs, especially for those selecting stocks with higher productivity.

**SUSCEPTIBILITY OF DIPLOID AND TRIPLOID OYSTERS, *CRASSOSTREA GIGAS*, TO *PERKINSUS MARINUS*.** Judith A. Meyers,\* Eugene M. Burreson, Bruce J. Barber, and Roger Mann, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The susceptibility of Pacific oysters, *Crassostrea gigas*, to the oyster parasite *Perkinsus marinus* was compared with that of eastern oysters, *Crassostrea virginica*, in two separate experi-

ments. Experiments were conducted in flow-through seawater systems with quarantined effluent. Oysters were challenged by the addition of infective *P. marinus* to the system. In the first experiment, which used only diploid oysters, 40% of the *C. gigas* became infected with *P. marinus* after 83 days, compared to 100% of the *C. virginica*. In the second experiment, which examined the susceptibility of diploid and triploid individuals of both species, prevalence was high in all groups after 60 days. In *C. virginica*, heavy and moderate infection intensities prevailed while *C. gigas* exhibited only light infections. Cumulative mortality of *C. virginica* after 150 days was 100% for the diploid group and 97.7% for the triploid group. Cumulative mortality of *C. gigas* after 150 days was 25.1% for the diploid group and 34.3% for the triploid group, but this mortality was not thought to be disease related. Thus, *C. gigas* was consistently more tolerant of *P. marinus* than *C. virginica*, and triploidy provided no increased tolerance for either species.

## CRAB STUDIES

**FUNCTIONAL ANATOMY OF THE COPULATORY APPENDAGES OF THE SNOW CRAB, *CHIONOECETES OPILIO* (O. FABRICIUS).** Peter G. Beninger,\* Département de biologie, Université de Moncton, Moncton, N.B., Canada, E1A 3E9; Robert W. Elner, Pacific Biological Station, Nanaimo, B.C.; Poussart, Yves, Département de biologie, Université de Moncton.

As part of ongoing studies of the reproductive processes in snow crab, the first and second gonopods of adult specimens were examined using scanning electron microscopy and histology. Surface features and setal types are described for both gonopods. The gonopods are highly modified compared to other brachyuran crabs and lower crustaceans. Suggestions for setal function are presented, based on size, orientation, location, fine structure, and cuticular insertion. Tubulation of the endopod is observed in both the first and second gonopods. Rosette glands are present in the basal half of the first gonopod but are absent in the second gonopod. A duct network from the rosette glands terminates in cuticular pores which are restricted to the ejaculatory canal, indicating that the rosette glands function in copulation, probably contributing to the seminal fluids. A mechanism for transfer of seminal fluids is proposed, based on the relative sizes of the gonopods and the morphology of the second gonopod.

**ABUNDANCE OF CANCER CRAB MEGALOPAE AND THE POTENTIAL RAMIFICATIONS TO POPULATION REGULATION.** Michael Clancy\* and J. Stanley Cobb, Department of Zoology, University of Rhode Island, Kingston, RI 02881.

The rock crab *Cancer irroratus* and Jonah crab *Cancer borealis*, are dominant members of the decapod community in the North Atlantic from Nova Scotia to the Mid Atlantic Bight. In this area, there has been considerable focus on the entire life history of

the American lobster, *Homarus americanus*, but the biology of *Cancer* larvae has been limited to only a few laboratory studies.

It appears that *Cancer* crab populations in Block Island Sound Rhode Island, may operate under different constraints and use different recruitment strategies than other commercially important crab species. Densities of *Cancer magister* and *Callinectes sapidus* megalopae are typically reported as 0.1/m<sup>3</sup>. We have routinely estimated *Cancer* megalopae in Block Island Sound to exceed hundreds/m<sup>3</sup> in the plankton, at least three orders of magnitude greater. Evidently, either fecundity is much higher in the North Atlantic *Cancer* species, or larval wastage (according to Reilly 1983 and Jamieson 1986) at the zoeal stage is low. The literature suggests that fecundity does not differ markedly among these species. Thus, the observed abundances must be a result of unequal larval mortality or some other physical or behavioral mechanism. The ramifications of these differences relative to population regulation will be discussed. This system may possess unique characteristics to allow us to assess the effects which variable larval delivery may have on the future composition of benthic populations.

**USE OF ARTIFICIAL COLLECTORS TO STUDY GROWTH OF SMALL RED KING CRAB.** William E. Donaldson\* and Susie Byersdorfer, and Forrest Blau, Alaska Department of Fish and Game, Kodiak, AK 99615.

Crab research biologists with the Alaska Department of Fish and Game have developed a methodology for collecting postlarval red king crab, *Paralithodes camtschaticus*, in their natural habitat. This methodology employs the use of artificial collectors constructed of a 1.8 m long section of tubular polyethylene plastic netting and stuffed with monofilament web. The collectors are set on longlines on the ocean floor and provide habitat for larval crab to settle on and metamorphose to a benthic existence. The primary goal of this research is to develop a method of quantifying settlement rates of red king crab and subsequent recruitment to the commercial fishery. Secondary goals are to define time of settlement and molt frequency and growth increments for small red king crab. Temporal sampling of collector gear for a one year period with the use of scuba divers, provided estimates of settling dates, molt frequency and growth increments. Fourteen samples of crab were collected from June 1, 1990 including the last larval stage (glaucothoe) and nine benthic instars.

**AN OVERVIEW OF CRAB MITIGATION IN GRAYS HARBOR, WASHINGTON.** Kay A. McGraw\* and David A. Armstrong, Fisheries Research Institute, University of Washington, Seattle, WA 98195; Fred C. Weinmann, Environmental Protection Agency, Region X, Seattle, WA; Walter H. Pearson, Battelle Marine Research Laboratory, Sequim, WA 98382.

Dredging associated with a navigation improvement project in Grays Harbor, WA resulted in the unavoidable mortalities of Dungeness crab (*Cancer magister* DANA). The U.S. Army Corps of Engineers judged the loss to constitute a significant impact and

began a small-scale mitigation program in April 1990. Oyster shell is being used to create habitat for juvenile (0+) crab, thus increasing survival and, eventually, offsetting impacts. To our knowledge the mitigation program is the first of its kind in the country in that it is for a commercial invertebrate species. The approach to mitigation was developed over several years and illustrates coordination, cooperation, and controversy among resource agencies and user groups and the role of scientific studies in the decision-making process. Although much data has been amassed on Dungeness crab in Grays Harbor, agency perspective on two other issues, the presence of eelgrass and possible dioxin contamination on potential mitigation sites, has caused major delays in full-scale mitigation and, possibly, increased costs. At issue in these cases is whether placement of shell on the intertidal impacts eelgrass (and to what extent shell constitutes habitat for a diverse fauna), and whether certain proposed mitigation sites are more likely to receive up-current contaminants than are other sites. Limited research on the latter two topics presents an interesting and difficult challenge for all concerned with the mitigation program.

**IMMIGRATION OF BLUE CRAB MEGALOPAE IN THE YORK RIVER, VIRGINIA: PATTERNS AND PROCESSES.** Eugene J. Olmi, III, The College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA, U.S.A. 23062.

Postlarvae (megalopae) of the blue crab (*Callinectes sapidus*) reinvade Chesapeake Bay after larval (zoeal) development in coastal waters. Proposed mechanisms for immigration include tidally-timed vertical migration, occupation of landward-flowing bottom water, transport by wind-induced water exchange between Chesapeake Bay and the adjacent shelf, or transport in surface slicks generated by internal waves. During three recruitment seasons (Jul–Nov 1987–1989), nightly abundance of megalopae in the plankton was highly variable, yet patterns of abundance were associated with the neap-spring tidal cycle and with wind events that affected transport near the mouth of Chesapeake Bay. These data suggest that blue crab megalopae were not reliant on a single transport process by which to gain entry to the estuary. In the York River, blue crab megalopae migrated vertically in response to light and tide, occupying surface waters during nocturnal flood tides. Thus, megalopae continued their migration up the estuary via tidal transport.

## PERKINSUS

**SUSCEPTIBILITY OF MSX-RESISTANT STRAINS OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, TO *PERKINSUS MARINUS*.** Eugene M. Burreson, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

As the first step in a selective breeding program to attempt to decrease the disease susceptibility of the eastern oyster, *Crassos-*

*Crassostrea virginica*, six strains were spawned and the progeny exposed to *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in the lower Chesapeake Bay. Three strains, a Delaware Bay MSX-resistant strain, a Delaware Bay native strain, and a Mobjack Bay native strain (lower Chesapeake Bay) were exposed for three summers; three other strains, a separate Delaware Bay MSX-resistant strain, a lower James River native strain (lower Chesapeake Bay) and a susceptible control strain were exposed for two summers. Both strains of MSX-resistant oysters developed by Rutgers University were highly susceptible to *P. marinus*. Cumulative mortality at the end of the study was 99% for both strains and growth virtually stopped after acquisition of *P. marinus*. Mean shell height did not reach market size in either MSX-resistant strain. All native strains (Delaware Bay, Mobjack Bay and James River) had about 80% cumulative mortality, mainly from *P. marinus*, but the Mobjack Bay strain also experienced moderate mortality from *H. nelsoni*. However, these strains grew well and survivors reached market size during the study period. The MSX-resistant strains offer little benefit in a selective breeding program for the Chesapeake Bay oyster industry because of their high susceptibility to *P. marinus* and poor growth, but the three native strains performed better and will be utilized, both as direct lines and as intraspecific hybrids, in a continuing selective breeding program to decrease the disease susceptibility of *C. virginica* stocks.

**EFFECT OF TEMPERATURE ON *PERKINSUS MARINUS* SUSCEPTIBILITY AND DEFENSE-RELATED ACTIVITIES IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*.** Fu-Lin E. Chu,\* Jerome F. La Peyre, and Carrie S. Burreson, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

Defense-related activities and infection of *Perkinsus marinus* in eastern oysters were examined under 4 temperature regimes, 10, 15, 20 and 25°C. Oysters were inoculated with trophozoites ( $10^6$  trophozoites oyster<sup>-1</sup>) of *Perkinsus* and maintained in a closed system. After six weeks, oysters were sacrificed and assessed for *Perkinsus* infections. Simultaneously defense-related activities of individual oysters from each test group including controls were measured. Results indicated that *Perkinsus* prevalence and intensity were correlated with temperature. Prevalence and intensity of *Perkinsus* infection was significantly lower in oysters maintained at 10°C than at higher temperatures. Total hemocyte count of oysters maintained at 25°C was significantly higher than all other temperature treatments. No significant difference was observed in granulocyte percentage between temperature treatments. At all temperature treatments, there was also a trend of decreasing total hemocyte count and percentage of granulocytes in oysters challenged by *Perkinsus* compared to controls. At 25°C, hemagglutinin level in *Perkinsus* challenged oysters appeared to be lower compared to control oysters.

**SEROLOGICAL PROBES FOR DETECTION OF *PERKINSUS MARINUS*: DEVELOPMENT AND APPLICATIONS.** Christopher F. Dungan, Maryland Department of Natural Resources, Cooperative Oxford Laboratory, Oxford, MD 21654.

*Perkinsus marinus* is currently the most destructive pathogen of eastern oysters, *Crassostrea virginica*, in the Gulf of Mexico and in Chesapeake Bay. Methods available for detection of this nonculturable protozoan pathogen require induction of hypno-spore formation by incubation of pathogen cells in Ray's Fluid Thioglycollate medium (FTM), and are not applicable to detection of dispersal stages in environmental water samples. Production of antibody probes which recognize all pathogen cell types was undertaken to enable the development of objective, rapid and quantitative diagnostic assays, and to permit definitive studies of disease epizootiology and pathogen life history.

Rabbits and mice were immunized with hypnospores harvested from FTM-incubated hemolymph of infected oysters. Rabbits produced high-titer specific antisera which recognized hypnospores bound to ELISA plates, and labeled all pathogen cell types in histological sections of infected oyster tissues. Immortal murine hybridomas secreting monoclonal antibodies which recognize hypnospores in ELISA assays were produced and cloned. Information on the binding specificities of serological probes, and on development of specific applications for them in diagnostics, epizootiology, and pathogen biology is presented.

***CRASSOSTREA GIGAS* DISEASE EXPOSURE TO *HAPLOSPORIDIUM NELSONI* AND *PERKINSUS MARINUS* IN CHESAPEAKE BAY WATERS.** C. Austin Farley, NOAA Cooperative Oxford Laboratory; Donna L. Plutschak and George E. Krantz,\* Maryland DNR Cooperative Oxford Laboratory, Oxford, MD 21654.

A population of native *Crassostrea virginica* collected from a low salinity habitat was diagnosed by hemanalysis to be free of *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* in the spring of 1989. A sample of 100 Japanese oysters, *Crassostrea gigas*, ranging from 45 to 105 mm, was received from Puget Sound, Washington, in May 1989 and diagnosed as above. Both populations were placed in a quarantined running seawater system at the Deal Island hatchery for natural exposure to both diseases over the summer. Both MSX and *Perkinsus* developed, intensified, and killed most of the native oysters. Japanese oysters did not show any MSX infections but did show low level infections of *Perkinsus* which did not intensify. In 1990, three groups of *C. gigas* seed produced by Rutgers University staff and a new population of native oysters were added to the original *C. gigas* population in the exposure system. By the fall of 1990, surviving native oysters were found to be 97% infected by *P. marinus* but showed no MSX. Only 14 of the original *C. gigas* survived and 5 animals exhibited low level *Perkinsus*. *Crassostrea gigas* seed ex-

perienced 90–99% mortality and did not grow in ambient salinity that ranged from 10.5 to 18 ppt.

**USE OF A HEMOLYMPH ASSAY TO DETERMINE SALINITY EFFECTS ON THE PROGRESSION OF *PERKINSUS MARINUS* DISEASE IN OYSTERS *CRASSOSTREA VIRGINICA*.** Julie D. Gauthier,\* Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550; William S. Fisher, EPA Environmental Research Laboratory, Gulf Breeze, FL 32561.

The intensity of disease caused by the endoparasitic protozoan *Perkinsus marinus* in oysters *Crassostrea virginica* can be diagnosed by hemolymph analysis. A completely quantitative hemolymph assay exhibited a significant linear relationship between the traditional semiquantitative tissue smear procedure in a recent study. Since the hemolymph assay does not require that oysters be sacrificed, disease progression within individuals oysters can be determined.

Hemolymph was sampled at two week intervals from oysters collected from ~25 ppt and maintained at  $25 \pm 2^\circ\text{C}$  in 6, 12, 18, 24, 30, and 36 ppt salinity. Samples were assayed for disease intensity, protein concentration, and lectin activity. Individual oyster analysis revealed that disease intensity was significantly positively correlated with time in 6, 30, and 36 ppt. Protein and lectin activity decreased significantly with time but not directly in relation to disease. Regression equations for the six disease progression curves are: 6 ppt  $y = 2.57 + 0.09x$  ( $r = 0.51$ ), 12 ppt  $y = 3.14 + 0.11x$  ( $r = 0.80$ ), 18 ppt  $y = 3.16 + 0.18x$  ( $r = 0.65$ ), 24 ppt  $y = 3.07 + 0.12x$  ( $r = 0.77$ ), 30 ppt  $y = 3.22 + 0.19x$  ( $r = 0.77$ ), and 36 ppt  $y = 4.01 + 0.27x$  ( $r = 0.94$ ) where  $x$  = time (weeks) and  $y$  = average  $\log_{10}$  hyphospore number/mL hemolymph. Our results indicate that *P. marinus* can progress in as low as 6 ppt and that the rate of progression increases with salinity.

**CHEMICAL INHIBITION OF *PERKINSUS MARINUS* IN AN *IN VITRO* TEST.** George E. Krantz, Maryland Department of Natural Resources, Cooperative Oxford Laboratory, Oxford, MD 21654.

A rapid diagnostic test for oyster parasites, recently developed at the Cooperative Oxford Laboratory, utilizes thioglycolate culture media in polystyrene tissue culture plates to detect *Perkinsus marinus* cells in circulating oyster hemolymph. This test was modified to serve as an *in vitro* assay system to detect compounds that exhibit inhibitory activity toward the enlargement of *P. marinus* cells in the thioglycolate media. The assay system detected 14 organic chemicals and 2 inorganic salts that had inhibitory activity. Cellular changes are described, and trypan blue vital stain confirmed that certain cellular changes resulted in death of the enlarging hyphospores.

**EFFECTS OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*: DISEASE DEVELOPMENT AND IMPACT ON GROWTH RATE AT DIFFERENT SALINITIES.** Kennedy T. Paynter,\* The Johns Hopkins University, Chesapeake Bay Institute, 4800 Atwell Rd., Shady Side, Maryland 20764, and Eugene M. Bureson, Virginia Institute of Marine Science, School of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

In order to assess the impact of *Perkinsus marinus* infection on oyster growth and mortality, juvenile oysters were raised in floating rafts at six sites around Chesapeake Bay. The sites were comprised of two low salinity sites, two moderate salinity sites and two high salinity sites. Oyster growth was monitored bi-weekly along with various water qualities including temperature and salinity. Condition index was measured monthly and disease diagnosis was performed bimonthly. Oyster growth was more rapid at higher salinities but was severely retarded by *Perkinsus* infection in the higher salinities where the parasite was more prevalent. Comparison of pre-infection and post-infection growth rates between sites showed that the reduction in growth rate was mitigated by lower salinity. Condition index was not related to salinity or site but was significantly reduced by infection. Reduction in condition, however, was not associated with increased mortality. Mortality was also less related to salinity or temperature than it was to previous infection. Groups which incurred heavy infection prevalences and intensities during their first year exhibited low mortality, while groups reinfected during the following year suffered heavy mortality. The results are discussed in relation to management and aquacultural practices and their relation to genetics and selective breeding of disease resistant oysters.

## ECONOMIC ANALYSIS FOR SHELLFISH ENTERPRISES

**SCALE ECONOMIES IN HARD CLAM AQUACULTURE.** Charles M. Adams, Food and Resource Economic Department, University of Florida, Gainesville, FL 32611; Robert S. Pomeroy, Department of Agricultural Economics and Rural Sociology, Clemson University, Clemson, SC 29361.

Interest in commercial hard clam (*Mercenaria mercenaria*) aquaculture has grown substantially during the past five years. This is particularly true for potential investors in the Southeastern U.S. Technological advances, as well as a growing domestic market for seafood, has enhanced the economic feasibility of culturing hard clams. Recent research funded by the National Coastal Resources Institute (NCRI) has examined the economic feasibility of culturing hard clams through the hatchery, nursery, and grow-out phases of production. The NCRI study assessed the profit-

ability of several methods of seed and market clam production, as stand-alone and integrated systems. However, the NCRI analysis failed to examine the effect scale economies have on the profitability of hard clam production. This paper will examine how the economic feasibility of hard clam culture is effected by changes in scale of operation, particularly for the hatchery and nursery components of the production process. The analysis will utilize basic financial statements, as well as partial budgeting, break-even, and sensitivity analyses, to assess the importance of scale economies to commercial hard clam aquaculture.

**WEST COAST GROUND FISH-SHELLFISH FISHERY INTERACTIONS: MANAGEMENT IMPLICATIONS, Susan S. Hanna**, Department of Agricultural and Resource Economics, Oregon State University, Corvallis, Oregon 97331.

The dynamics of West Coast fisheries are complex and variable. Interactions between fisheries are common. An important multi-fishery interaction exists between groundfish, crab, and shrimp fisheries. These interactions are complex and pose difficult management questions related to the timing of seasons, state-federal interactions, market interactions, the impact of regulatory actions, and multi-fishery system management.

This paper first presents an historical overview of the development of interactions in groundfish, crab, and shrimp fishing, showing changes in the diversity of fishing activities over time. The progress of management of these fisheries, including state-federal interactions, is presented next. Key factors linking the three fisheries are outlined, including market prices, investment costs, fishing effort, and management actions. Current management issues facing these three fisheries are discussed, with proposals for rationalizing a system approach for groundfish-shellfish management.

**DEVELOPMENT AND APPLICATION OF A BIOECONOMIC MODEL FOR THE OYSTER SEED FISHERY OF BARATARIA BAY, LOUISIANA. Earl J. Melancon, Jr.,\*** Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310; **Richard Condrey**, Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803.

A three year study of eight oystermen bedding oyster seed on 19 leases determined the influences of water temperature and salinity on lease production. In turn, lease production influenced fuel, labor and other operating expenses in the fishery. The data was developed into a bioeconomic model of the Bay fishery.

Application of the model predicts that lease production can be increased significantly if oystermen wait until October to be bed their seed instead of in September, their traditional time to begin. Additionally, production increases significantly if all oysters are harvested by the following mid-summer to minimize predator and disease problems.

The potential production advantage of bedding seed in late fall and harvesting before the following late summer is not a new con-

cept. However, this bioeconomic model quantifies for the first time the synergistic influences of environment and fishing methods with an oysterman's costs to operate in a Louisiana oyster fishery.

**ECONOMIC FEASIBILITY OF FLOATING RAFT OYSTER CULTURE IN CHESAPEAKE BAY. Kennedy T. Paynter,\*** The Johns Hopkins University, 4800 Atwell Rd., Shady Side, MD 20764, and **Samuel H. Shriver**, World's End Aquaculture, 2324 W. Joppa Rd., Lutherville, MD 21093.

Previous research has shown that oysters grow very rapidly in floating trays in Chesapeake Bay. In order to assess the economic feasibility of floating raft culture in Chesapeake Bay, 400,000 cultchless spat (10 mm) were purchased from a local hatchery, grown to market size (76 mm) and sold. Capital equipment, supplies, hours and type of labor, and support equipment and activities were carefully recorded. This information provided a basis for the estimation of the cost of oyster production using floating raft culture.

The production site was located on the Wye River, MD. Previous research had shown that the site supported good growth rates and had a history of low disease prevalence. The animals were introduced in weekly batches of 100,000 during September, 1989. Spat were initially maintained in closed 3 mm mesh cages to protect them from mud crabs and moved into sequentially larger mesh cages and trays as they grew. Twelve months later, when the animals reached 50–60 mm in length, they were moved from the Wye River to Mobjack Bay, VA where they were "finished." Finishing was comprised of a two to four month tray culture period in which the animals grew an additional 25 to 30 mm and acquired a saltier taste for marketing. The animals were sent directly to market from Mobjack Bay.

Depending on the manner of capital expenditure treatment, the cost of raising oysters in floating raft culture was estimated at \$0.13–0.19/oyster. This estimate was calculated as the sum of labor, capital, supply and ancillary expenditures necessary to grow to market size an estimated 150,000 oysters. The relative costs of oyster production, alternative culture methods and the market value of oysters cultured in floating rafts will be discussed.

**OYSTERS LEASE TRANSFERS, AUCTIONS, AND LENDING: ROLES IN REHABILITATION OF LOUISIANA'S OYSTER INDUSTRY. Kenneth J. Roberts,\*** Office of Sea Grant Development, L.S.U., Baton Rouge, LA 70803; **Walter R. Keithly**, Coastal Fisheries Institute, L.S.U., Baton Rouge, LA 70803.

U.S. oyster landings have been declining for the past several decades and the decline has been particularly pronounced since the mid 1980's. While much of the rehabilitation and expansion emphasis, in both practice and theory, has been public ground oriented, private grounds can also provide the catalyst for much of the rehabilitation and expansion efforts. In states like Louisiana,

where the majority of production is derived from these private grounds, it is logical to target this component of the harvesting sector for rehabilitation effort. The information void regarding economic and financial aspects of the leasing situation in the primary leasing states is seen to be one of the major obstacles confronting the oyster industry and management authorities in the planning stages of rehabilitation and expansion projects. An analysis of the stability of lease-based businesses is a precursor to reef rehabilitation efforts of privately owned grounds. While results are specific to Louisiana, information contained in the report should be of use to other states throughout the Southeast in planning their respective rehabilitation activities. The transfer of oyster leases was shown to be increasing. This was shown to involve increasing lease size during 1950–89. Aggregating adjacent leases was discovered. This could facilitate economies for private rehabilitation investments. Leases returned to the state for various reasons have been auctioned since 1987. The acres, location, minimum bid, and successfully bid acreage were depicted to identify the preferred locations for private rehabilitation efforts. The lease length and renewal rights were found to be important in obtaining loans for rehabilitation.

**ECONOMICS OF FRESHWATER *CHERAX* LOBSTER DEVELOPMENT.** Michael C. Rubino, Bluewaters, Inc., 4350 East West Hwy, Suite 600, Bethesda, MD 20814.

Freshwater lobsters of giant crayfish of the genus *Cherax*, indigenous to Australia, are promising new aquaculture species. Fast growth—semi-intensive pond culture, low feed costs, high meat yield, and ready markets provide these species with the biological, physical, and market characteristics necessary for a profitable aquaculture venture. While culture of three *Cherax* species is underway in Australia, adapting the culture to other environments requires years of research and development. A variety of factors such as *Cherax* biology, aquaculture methods, environmental considerations, and production economics influence the direction of the development process. This article examines the economic factors and considerations in marron (*Cherax tenuimanus*) and red claw (*Cherax quadricarinatus*) research, pilot projects, and commercialization in the United States and the Caribbean. A production economics model that describes the relationships between yield (growth and survival), market price, and fixed and variable costs can help project managers make critical decisions about culture methods, research and development priorities, and scale of commercial operation.

**MARINE AQUACULTURE ENFORCEMENT: ARE PUBLIC AGENCIES PASSING THE BUCK?** Eric M. Thunberg, Food and Resource Economics Department, 1170 McCarty Hall, University of Florida, Gainesville, FL 32611.

All states on the Eastern seaboard regulate aquaculture enterprises for several purposes. Similarly all states regulate their wild fisheries as well. In some instances the regulatory authority over

aquaculture and wild fisheries overlap. Minimum size regulations for harvested shellfish are one such example. In several states aquaculture enterprises may be exempted from any harvest size restrictions. However, in some states, Florida being one of them, aquaculture enterprises are not exempted from harvest size restrictions under certain conditions. The principle argument supporting maintenance of size restrictions for wild and cultured shellfish is that marine enforcement cannot distinguish between wild and cultured product. Thus, in the interest of protecting public resources, a minimum size restriction is placed on all product regardless of its origin.

Harvest restrictions may cause financial losses to aquaculture entrepreneurs in terms of mortalities experienced while waiting for the product to reach a legal size and in terms of increased interest costs. Therefore, private entrepreneurs are forced to bear the burden of the cost of enforcing harvest size limits that are designed to protect public resources. This paper discusses this issue in more detail and presents simulated estimates of financial losses due to harvest size restrictions for a hypothetical oyster aquaculture business. Estimates of reduced revenues, increased capital costs, and aquaculture firm rates of return are presented and alternative means for marine aquaculture enforcement are suggested.

## OYSTER RECRUITEMENT IN THE MID-ATLANTIC STATES

**SPATIAL VARIATION IN MAGNITUDE AND TIMING OF SETTLEMENT OF LARVAL OYSTERS, *CRASSOSTREA VIRGINICA*.** Bruce J. Barber,\* Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062; S. R. Fegley, Shellfish Research Lab, Rutgers Univ., P.O. Box 687, Port Norris, NJ 08349; R. I. E. Newell, Horn Point Labs, Univ. of Maryland, P.O. Box 775, Cambridge, MD 21613.

The timing and magnitude of settlement of larval oysters, *C. virginica*, on spat collectors were examined both within and between estuarine systems in Delaware Bay and upper and lower Chesapeake Bay. Data collected between June and October of 1981 through 1990 from three stations representing the upper (Deepwater Shoal), middle (Wreck Shoal), and lower (Naseway Shoal) James River, VA, indicate that: 1) annual settlement totals increased in a downriver direction and tended to be greater in alternate (odd numbered years), with 1985 having the greatest settlement; and 2) for the 10 year period, an average of 61%–69% of total annual settlement (increasing in a downriver direction) occurred during the 5 week period from 13 July to 16 August. Comparable data from upper Chesapeake Bay (MD) and Delaware Bay (NJ) are being similarly examined so that possible mechanisms regulating the timing and magnitude of larval settlement both within and between estuarine systems can be discussed.

### **EFFECTS OF TIDAL POSITION AND SUBSTRATE ON SPATFALL IN SOUTHEAST U.S. OYSTER POPULATIONS.**

**M. P. Crosby\*** and **P. K. Kenny**, Baruch Marine Lab, U of South Carolina, Georgetown, SC; **V. G. Burrell, Jr.**, Marine Resources Research Institute, SCWMR, Charleston, SC.

In an attempt to elucidate possible differences between inter- and subtidal oyster spat availability, we analyzed spatfall data from two independently conducted studies. The study area included 16 total sites with 11 dominated by inter-, and 5 dominated by subtidal adult oyster populations. Shell-string collectors used from 1972–1975 at sites dominated by both inter- and subtidal adult populations, while asbestos collecting plates were used from 1982–1990 at sites dominated by intertidal adult populations only. Although the two studies were conducted at different times, the same general temporal trends in spatfall were observed regardless of substrate or site. Although the majority of set occurred from May to September irrespective of site or collector substrate, shell-string collectors picked up spatfall in all months except March and December, while asbestos plate collectors had set from May through November. Shell-string collectors also demonstrated consistently greater subtidal than intertidal spat set, regardless of time of set or tidal height of the adult populations, and exhibited a mono-modal annual peak in set occurring in mid-summer. Conversely, the asbestos plate collectors tended to demonstrate bimodal annual peaks in set occurring in late spring and late summer with no clear preference demonstrated between subtidal and intertidal positions. The atypical sites that have historically been dominated by subtidal adult populations always exhibited an order of magnitude lower set (<20 spat/M<sup>2</sup>/day) than areas dominated by intertidal adult populations (>400 spat/M<sup>2</sup>/day), regardless of spat collector tidal height. This low set may be a contributing factor for subtidal adult populations being dominated by low density "singles" while intertidal adult populations occur as high density "clumps." The dominance of intertidal populations of adult oysters in the southeastern U.S. may also be influenced by low availability of larvae in subtidal waters.

**RELATIONSHIPS BETWEEN SPATIAL AND TEMPORAL PATTERNS OF SUCCESSIVE LIFE-HISTORY STAGES IN THE OYSTER *CRASSOSTREA VIRGINICA*.** **Stephen R. Fegley\***, Rutgers Shellfish Res. Lab., POB 687, Bivalve, NJ 08349; **R. I. E. Newell**, HPEL, Univ. Md., Cambridge, MD 21613; **B. J. Barber**, VIMS, Gloucester Point, VA 23062.

For 36 consecutive years the spatial and temporal patterns of abundance of: 1) oyster larvae, 2) larval settlement (spatfall), and 3) oysters 3 mo. old and older have been followed in Delaware Bay. The three types of samples were taken, respectively, by: 1) filtering water samples collected weekly 1 m below the water surface and 1 m above the bottom, 2) counting spat that have settled onto clean shell placed in the field weekly, and 3) oyster dredge samples taken on the oyster reefs in the late fall and winter of the

year. Comparable records from the Chesapeake exist for several years as well. We examine the annual trends in these data sets to determine what relationships, if any, occur among successive life-history stages. Preliminary inspection of the data reveals little association between oyster success in one life-history stage and any preceding stages. Possible explanations for the lack of spatial and temporal connections of the data sets will be discussed. This work was supported by NJDEP and NJAES funds and is Inst. of Mar. & Coastal Sci. contribution #91-07 and NJAES publication #K-32406-1-91.

**LONG-TERM TRENDS IN OYSTER RECRUITMENT ON NATURAL BARS IN THE MARYLAND PORTION OF THE CHESAPEAKE BAY.** **George E. Krantz**, Maryland Department of Natural Resources, Cooperative Oxford Laboratory, Oxford, MD 21654.

A data set of spat counts on bottom material sampled in the fall of each year from 1939 to 1989 provides a description of annual variation, spatial differences, and long-term trends in oyster recruitment on natural bars in the Maryland portion of the Chesapeake Bay. "Key bars" selected in 1975 have been monitored to the present time for spatfall and compared to historical observations. The arithmetic mean and 5-year moving average depict a central tendency, with major periods of depressed recruitment from 1950 to 1960, 1969 to 1980, and from 1978 to the present. Geographic distribution of recruitment varied widely, with a strong indication that low salinity environments are now receiving lighter spatfall than in the initial years of the data set. The number of oyster bars that receive light spatfall have increased disproportionately over time.

**VARIABILITY IN THE RELATIONSHIP BETWEEN LARVAL SETTLEMENT AND RECRUITMENT IN POPULATIONS OF THE OYSTER *CRASSOSTREA VIRGINICA*.** **Roger I. E. Newell\***, Horn Point Environmental Laboratories, University of Maryland, Cambridge, MD 21613; **B. J. Barber**, Virginia Institute of Marine Science, Gloucester Point, VA; **S. R. Fegley**, Shellfish Research Laboratory, Port Norris, NJ 08349.

Various types of settlement substrates suspended off-bottom have been used by numerous investigators to monitor the timing and intensity of juvenile (= spat) oyster settlement. Using historical data from the lower and upper portions of Chesapeake and Dealware Bays we examine the utility of these off-bottom collector substrates as a method of predicting the magnitude of spat recruitment to natural oyster populations. Based on data from the upper Chesapeake Bay it appears that spat abundance on natural oyster beds in the Fall was up to 99.9% lower than predicted from larval settlement on adjacent off-bottom collectors, integrated over the entire reproductive season. Results from predator exclusion cage work at this one location over two summers demonstrated that this discrepancy was principally due to high predation

rates on <7 mm spat by benthic micropredators, such as flatworms (*Stylochus ellipticus*).

**MEASURING OYSTER SPATFALL: A COMPARISON OF METHODS.** **Sonia Ortega**,\* Duke University Marine Laboratory, Beaufort, NC 28516; **Steve Fegley**, Shellfish Research Laboratory, Rutgers University, P.O. Box 687, Port Norris, NJ 08349; **Roger Newell**, Horn Point Environmental Laboratories, P.O. Box 75, Cambridge, MD 21613; **Bruce Barber**, Virginia Institute of Marine Sciences, Gloucester Point, VA 23062.

The above authors have been using different methods to estimate spatfall in the eastern oyster *Crassostrea virginica* along the East coast of the United States. In order to establish compar-

isons among regions and to plan future cooperative monitoring efforts, it was necessary to determine which method provided the most accurate data. During the summer of 1990, each author deployed for different types of collectors at sites previously used for spatfall studies. Sites were located in the eastern half of Delaware Bay, upper and lower Chesapeake Bay and Core Sound, North Carolina. Collectors used were oyster shells in bags, oyster shells in strings, oyster shells tied to vexar mats and asbestos plates. Spatfall was measured at weekly intervals for six weeks. Spatfall was greater on the plates than on the other collectors, although the efficiency of each collector varied in space and time. There was no statistically significant difference in the amount of variation within each collector type.



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**COVER PHOTO:** Developmental sequence of hatching lobster (*Homarus americanus*) larvae. From bottom left: Blue egg; prelarva (outer chorion split); prelarva with inner chorion; prelarva without chorion; molting prelarva; stage I. Photo by Patricia Biesiot, Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi, 39406-5018.

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### REGINALD VAN TRUMP TRUITT

Dr. Reginald Van Trump Truitt, pioneering oyster biologist and founder of the Chesapeake Biological Laboratory, died on April 11, 1991 at the age of 100. He was born on August 12, 1890 in Snow Hill, Maryland into a family of oyster planters. He received his BS (1914) and MS (1924) degrees from the University of Maryland, and his Ph.D. (1929) from American University. In 1920, he joined the faculty of the University of Maryland as an Assistant Professor, moving through the ranks to become Professor of Zoology and Aquiculture [sic] in 1925. In 1942, he became Director of the Maryland Department of Research and Education, a position he held until his retirement in 1954. He lived in retirement on Kent Island and devoted time to Maryland history, writing a number of accounts about Kent Island, Maryland's coastal hurricanes, Assateague Island, the ospreys of Great Neck, etc. He also worked to have Assateague Island recognized as a national seashore.

Dr. Truitt began his academic career as a student of insects, but turned his attention to oysters because of his concern about the decline in oyster harvests in Chesapeake Bay. Beginning in 1918, he spent summers on Solomons Island where he began investigations into oyster spawning and setting. He recognized the importance of environmental factors, so measured temperature, specific gravity, and pH of the water while sampling oyster larvae over major producing areas in Maryland. This work entailed travel over 500 km of water on a regular basis. He determined by 1927 that water quality was satisfactory for oyster production, but that only a few of the oyster beds studied in 19 regions of Maryland harbored enough larvae to justify the effort and cost of shell planting. He also noted high inter-annual and inter-regional variability of oyster larval abundances.

From 1927–1929, he and his colleagues concentrated on 10 oyster bars, making field trips nearly every week over 200 km of distance to collect larvae and measure water quality variables. Effort centered on the relationship of broodstock abundance and larval abundance, setting and spat survival, comparisons of settlement on different kinds of cultch, the appropriate time to plant shells, and the physical nature of formerly productive oyster beds. He demonstrated that oyster shell was more attractive to settling oyster larvae than glass, wood, bricks, cinders, and pebbles. He found correlations between broodstock abundance on an oyster bed and larval abundance in the overlying water column, noting however that high numbers of broodstock alone did not assure high incidences of spat settlement in all regions of the Bay. His recommendations that the minimum harvestable size of oysters be increased from 2½" to 3" and that shell and brood oysters be used to rehabilitate depleted oyster grounds were enacted into Maryland law in 1927. Thus, in that year, a shell planting program partly financed by a gas tax on work boats and a requirement that 10% of shucked shell be made available by oyster packing houses to the State as cultch were implemented.

Ever the practical biologist, Dr. Truitt established an experimental "oyster farm" in 1931 on a 1000-acre reserve in the Honga River, an estuarine tributary in central Chesapeake Bay. He had found the region to have numerous larvae in the water although the oyster

## IN MEMORIAM: REGINALD VAN TRUMP TRUITT



grounds had been badly overfished, with limited shell available. Over a three-year period he had 212,000 bushels of shell planted on one 50-acre section. About 4,000 bushels of seed oysters were harvested in autumn 1934 from a four-acre patch within the planted area. He estimated that about 50,000 bushels had set where oysters had not been produced for years, demonstrating nearly 60 years ago the aquacultural potential of properly managed shell planting in Maryland.

Much of Dr. Truitt's research was not supported by outside funds until the 1930's, and depended on his own resources, and on donated sampling gear, chemicals, work space, and laboratory assistance (Maryland's Conservation Department did provide manned boats and supported the field program in later years). Nevertheless, he produced many technical reports in the Annual Reports of Maryland's Conservation Department, as well as an illustrated guide to oyster biology and Maryland's oyster industry. Initially, Dr. Truitt's summer work was performed in the Episcopal parish hall on Solomons Island. In 1925, he established the Chesapeake Biological Laboratory, with construction of its first building begun in 1931 with the assistance of the Conservation Department. Cooperating institutions at its inception were the Carnegie Institution, the University of Maryland and the Johns Hopkins University, and Goucher, St. John's, Washington, and Western Maryland Colleges. Thus began what is now the oldest state-supported marine laboratory in continuous operation on the eastern seaboard. In 1929, five students (three men, two women) were admitted to the laboratory to study and perform research, four from the U.S. and one from Columbia, South America. This tradition of summer interns working at CBL (and later at its sister laboratory Horn Point Environmental Laboratory) has continued to this day.

Dr. Truitt was active in many scientific societies, including AAAS, AFS, ASLO, and ASZ. He was Vice-President of the Ecological Society of America in 1950-51, and was made a Fellow of the Maryland Academy of Sciences. Of significance to members of the National Shellfisheries Association are his serving as President of NSA in 1935 and 1936, and his recognition as an Honorary Life Member in 1959. As noted earlier, Dr. Truitt had a strong interest in historical matters. From 1952 to 1962 he founded four county historical societies in Maryland (Worcester, Calvert, Queen Anne's and Charles counties). Always an athletic man, he represented the University of Maryland in lacrosse as an undergraduate (playing against Jim Thorpe in 1913) and coached the University's team to its first National Championship in 1925. He entered the Lacrosse Hall of Fame in 1960 and the University of Maryland Athletic Hall of Fame in 1984.

Although the oyster harvests in Chesapeake Bay have continued their decline (mainly due to politically influenced management, overfishing, pollution and, most recently, disease), many shellfish biologists have long believed that, had Dr. Truitt's recommendations of 60 years ago been applied rigorously by the State, harvests would now be higher, and oyster farming rather than oyster hunting might be a present reality in Maryland. In 1981, Dr. Truitt received the Rachel Carson Award for his work to preserve the Chesapeake Bay and its environment. His life work and accomplishments point to a significant lesson. Dedicated individuals must struggle to get their scientific and conservation messages across to politicians, managers, and the lay public, and must often be disappointed by the inertia of the system. Yet they persist, to the benefit of future researchers and society. If we can make a fraction of the contribution to science and society that Dr. Truitt made in his time, we will have done well.

Victor S. Kennedy  
Horn Point Environmental  
Laboratory  
Cambridge, Maryland

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# MORTALITIES OF IMPOUNDED AND FERAL MAINE LOBSTERS, *HOMARUS AMERICANUS* H. MILNE-EDWARDS, 1837, CAUSED BY THE PROTOZOAN CILIATE *MUGARDIA* (FORMERLY *ANOPHRYS* = *PARANOPHRYS*), WITH INITIAL PREVALENCE DATA FROM TEN LOCATIONS ALONG THE MAINE COAST AND ONE OFFSHORE AREA.

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**ABSTRACT** The first recognition that the ciliate *Mugardia* was a potential pathogen in lobsters from U.S. waters was on April 9, 1990 when a recently dead 88 mm female lobster, received for examination from a holding pound on Southport Island, Maine, was negative for gaffkemia (*Aerococcus viridans*), but showed numerous ciliates in stained hemolymph and hepatopancreas preparations. Further submittals from this pound and from a pound at Small Point, Me. showed that ciliates were responsible for serious lobster mortalities at these two facilities. Two transmission experiments were conducted, one commencing April 13, 1990, the other May 8, 1990. In both instances, when hemolymph from ciliate-infected lobsters was injected into uninfected lobsters, the lobsters became positive for ciliate disease and the disease progressed in severity to death of the lobsters. Review of lobster hemolymph and hepatopancreas slides from past years revealed that *Mugardia* was present in lobster hepatopancreas but unrecognized as a potential pathogen in Maine lobsters dating back to 1980. Ciliates were found to be endemic to feral lobster populations and during this study were responsible for mortalities of freshly-caught lobsters from the Pemaquid, Maine fishery. Prevalence data and intensity of infections in individual lobsters are given for ten locations along the Maine coast and one offshore area. Histological sections revealed that ciliates were responsible for tissue destruction, especially of the intestine.

**KEY WORDS:** lobsters, *Homarus americanus*, protozoan ciliate *Mugardia* (formerly *Anophrys* = *Paranophrys*), mortalities, Maine.

## INTRODUCTION

Holotrichous ciliates of the genus *Anophrys* (now *Mugardia*) have been associated with mortalities of various species of both laboratory-held and feral crustaceans since first described by Cattaneo (1888) in the hemolymph of the green crab, *Carcinus maenas* Linnaeus, from Venice, Italy.

Additional reports of *Mugardia* in crabs and an isopod have been from France and the West Coast of the United States: Poisson (1930) in *Carcinus maenas* from France; Bang et al. (1972) in *Cancer pagurus* Linnaeus from commercial holding tanks in Brittany, France; Armstrong et al. (1981) in laboratory-held dungeness crabs, *Cancer magister* Dana from Newport, Oregon; Sparks et al. (1982) in a laboratory-held and a feral dungeness crab, *Cancer magister* from Washington and Hibbits and Sparks (1983), in feral isopods, *Gnorimosphaeroma oregonensis* Dana, from the Kodiak region of Alaska. These microscopic ciliates can be lethal to their hosts, multiplying by binary fission to massive numbers and destroying not only hemocytes but their hosts' tissues (Armstrong et al. 1981).

There have been two published reports of these ciliates occurring in American lobsters, *Homarus americanus* H. Milne-Edwards, both from Atlantic Canada. Aiken et al. (1973), reported a ciliate, presumed to be an *Anophrys* species, to be responsible for causing significant mortalities of *Homarus americanus* from the Canadian fishery being held in flow-through holding tanks at St. Andrews, New Brunswick in the winters of 1971–1972 and 1972–1973. Lobsters died within six weeks as a result of the ciliates reaching high densities in the hemolymph and destroying the hemocytes. Aiken and Waddy (1986) reported finding this blood ciliate in wild lobster stocks three times in 14 years, all cases also occurring during the winter.

Armstrong et al. (1981), stated that holotrichous ciliates found in

crustaceans which have been historically assigned to the genus *Anophrys* are now placed in the genus *Paranophrys*. This lobster ciliate, considered to belong to the genus *Anophrys* by Aiken et al. (1973), Aiken and Waddy (1986) and to the genus *Paranophrys* by Sindermann (1990), has recently been reclassified by the Society of Protozoologists to the class Oligohymenophorea, subclass Hymenostomata, order Scuticociliatida, family Paranophryidae and genus *Mugardia* (Loughlin and Bayer 1991).

This is the first report of these ciliates inhabiting lobster livers (hepatopancreas). This report shows that *Mugardia* was responsible for serious mortalities in Maine holding pounds, that it was present in lobster hepatopancreas but unrecognized as a potential pathogen in Maine lobsters dating back to 1980, that it can be found in the hepatopancreas when it is not evident in the hemolymph, that it can be transferred from lobster to lobster, that ciliates are endemic to feral lobster populations and during this study were responsible for mortalities of freshly-caught lobsters from the Pemaquid, Maine fishery.

Prevalence data and intensity of infections in individual lobsters are given for ten locations along the Maine coast and one offshore area.

We also provide evidence to suggest that these ciliates occur in feral rock crab, *Cancer irroratus* Say, populations.

## MATERIALS AND METHODS

All lobsters included in this study were from the Maine fishery. Wild lobsters used in this study were obtained from another project within the Department of Marine Resources. A total of 89 feral lobsters, ranging in carapace length from 81.6–124.8 mm were received between November 7 and December 8, 1990 from ten locations along the Maine coast and one offshore area. These locations included Scarborough, Phippsburg, Pemaquid, Tenants Harbor, Stonington, Jonesport, an area south of Little Machias Bay,

Cutler (in the channel near Machias Seal Island), Cutler (near shore), Lubec-Trescott and the NE portion of Jordan Basin (75 miles offshore, SSE of Jonesport, Maine in 117 fathoms).

Industry members experiencing mortalities in their holding pounds submitted 20 market-size lobsters, ranging in carapace length from 82–102 mm, between April 9 and May 23, 1990. In addition, industry submitted two juvenile lobsters of 20 and 31 mm carapace length and one rock crab, *Cancer irroratus*, of 91 mm carapace width on May 10. On May 16 seven green crabs, *Carcinus maenas* Linnaeus, ranging in carapace width from 55–79 mm and seven rock crabs ranging in carapace width from 90–115 mm were submitted.

Captive lobsters used in transmission experiments were donated by DMR's lobster project.

Lobsters were measured for carapace length, sexed and gross examined for evidence of recent or old wounds, shell disease, external parasites or other abnormalities. A piece of gill and swimmeret were removed and examined at 100 $\times$  and 200 $\times$  for evidence of live ciliates within the vascular spaces of the gill or swimmeret.

Hemolymph samples were obtained from the ventral segmented abdomen via a sterile, disposable hypodermic syringe and a 21 gauge needle. A drop of hemolymph was placed upon a culture slide, covered with a cover glass and examined at 100 $\times$ , 200 $\times$ , and 1000 $\times$  for evidence of live ciliates and bacteria. In addition, a drop of hemolymph was placed on a standard 3  $\times$  1 inch microscope slide and, with the aid of a second slide, the smear was made and allowed to air-dry. The hemolymph smear was then fixed for three minutes in absolute methanol and stained with a giemsa solution containing one part stock giemsa to ten parts of distilled water for seven minutes. Microscopic examinations of stained hemolymph slides were made at 40 $\times$ , 100 $\times$ , 200 $\times$ , and 1000 $\times$  for evidence of ciliates, gaffkemia or other entities.

Hepatopancreas samples were obtained by dissecting a small piece of tissue from several areas of the organ, placing the excised pieces on a microscope slide and spreading the tissue into a thin film. Smears were fixed, stained and examined in the same manner as the hemolymph smears. In some instances portions of hepatopancreas remained intact on the slide and upon microscopic examination ciliates were seen in clusters rather than evenly distributed.

Live ciliates were demonstrated in hepatopancreas tissue by excising a small piece of hepatopancreas, teasing it into a culture slide, adding several drops of water and examining the resulting slurry at 100 $\times$ , 200 $\times$  and 1000 $\times$ .

Hemolymph samples were drawn from a leg of rock and green crabs via sterile disposable hypodermic syringes and needles. Live and stained hemolymph preparations were made in the same manner as for lobsters.

Lobsters used in transmission experiments were held in a tank with a flow-through water system. Short lengths of PVC pipe were placed in the tank for shelter for the lobsters. Captive animals were fed crushed soft-shell clams and previously frozen herring. Donor hemolymph for transmission experiments was withdrawn from the ventral segmented abdomen, recipients were injected just below the carapace in the area of the pericardial sinus.

Selected tissues were removed, fixed in Davidson's solution and processed using routine histological methods. Tissues were embedded in paraffin, sectioned on the microtome at 7 $\mu$ m and stained with hematoxylin and eosin stains. Following coverslip-

ping, examinations were made at 40 $\times$ , 100 $\times$ , 200 $\times$ , 400 $\times$  and 1000 $\times$ .

## RESULTS

### *Southport Island, Maine*

Our first indication that ciliates were a potential pathogen in Maine lobsters was on April 9, 1990 when a recently dead 88 mm female lobster, received for examination from a holding pound on Southport Island, Maine was negative for gaffkemia, (*Aerococcus viridans*), but showed numerous ciliates in stained hemolymph (blood) and hepatopancreas (liver) preparations (Figs. 1, 2). This pound, with a rated capacity of 100,000 pounds of lobsters and reportedly stocked with 110,000 pounds, had been experiencing serious mortalities and lobsters were not transporting well, even to distances as close as 60 miles.

Further submittals of lobsters from this pound on April 11 (2 live, 1 moribund, 2 dead), April 12 (2 live), April 13 (4 dead) and May 10 (2 live, 1 dead) were all negative for gaffkemia but showed ciliates in unstained fresh hemolymph preparations from nine of fourteen lobsters, and in stained hemolymph preparations from ten of fifteen lobsters and in both lobsters from which hepatopancreas smears were prepared. The one stained hepatopancreas preparation from #2 (97 mm male) dead lobster of April 13 not only showed ciliates of the same general form, but more degenerated forms than in the hepatopancreas smear of the dead lobster submitted on April 9 (Fig. 3). Concentrations of ciliates in these further submittals ranged from moderate to numerous in fresh unstained hemolymph and in the hepatopancreas preparations and few to numerous in stained hemolymph preparations (Table 1). Hemolymph containing numerous ciliates sometimes appeared milky white in color and blood cells (hemocytes) were scarce, similar to that reported by Bang et al. (1972) in *Cancer* crabs from commercial holding tanks in France and Aiken et al. (1973) in *Homarus americanus* from holding tanks at St. Andrews, N.B., Canada.

Live ciliates varied in size and were generally elongate to pyriform in shape with a rounded posterior end and a bluntly tapered anterior end, but they would frequently change shape as they moved through the hemolymph and negotiated among themselves. A posterior contractile vacuole was apparent in many instances

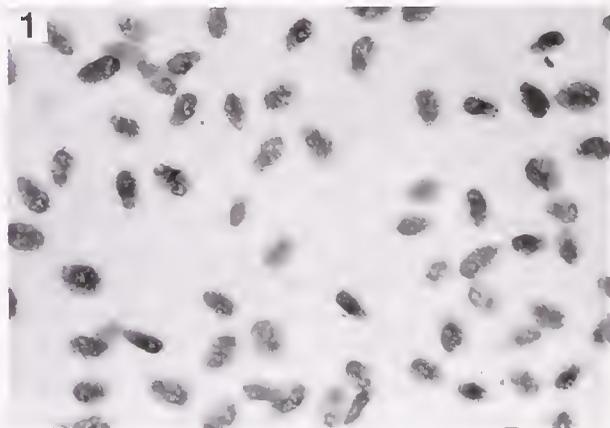


Figure 1. Numerous ciliates in the stained hemolymph smear from the recently dead 88 mm (carapace length) female lobster submitted April 9, 1990 from the impounding facility on Southport Island, Maine.

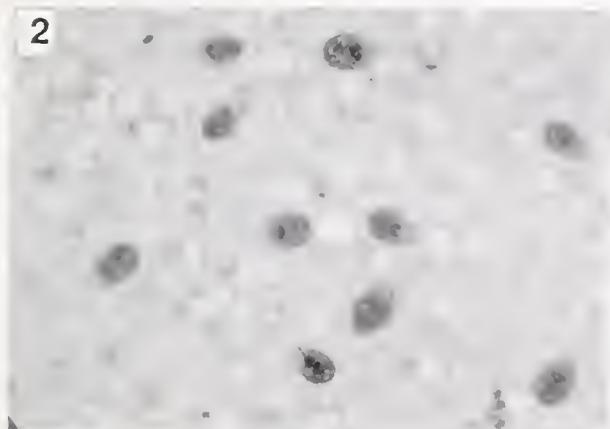


Figure 2. Ciliates in the stained hepatopancreas smear from the recently dead 88 mm female lobster submitted April 9, 1990 from the impounding facility on Southport Island, Maine, showing forms similar to those found in the hemolymph.

(Fig. 4). The cilia propelling these parasites were readily seen at 200 $\times$ . These ciliates were apparently of the *Anophrys* (now *Mugardia*) type, reported by Aiken et al. (1973) to have caused severe mortalities in lobsters being held in flow-through holding tanks at St. Andrews, New Brunswick during the winters of 1971–72 and 1972–73.

Eight rock crabs, *Cancer irroratus*, and 7 green crabs, *Carcinus maenas*, were trapped in the Southport Island pound and examined for evidence of ciliates. On May 10, one rock crab, 91 mm in carapace width, was negative for ciliates. However, on May 16, a 95 mm male, one of seven rock crabs 90–113 mm in carapace width sampled, showed a ciliate in a fresh hemolymph preparation, but not in the stained hemolymph smear. Seven green crabs, 55–79 mm in carapace width, trapped on May 16, were negative for ciliates in fresh and stained hemolymph preparations.

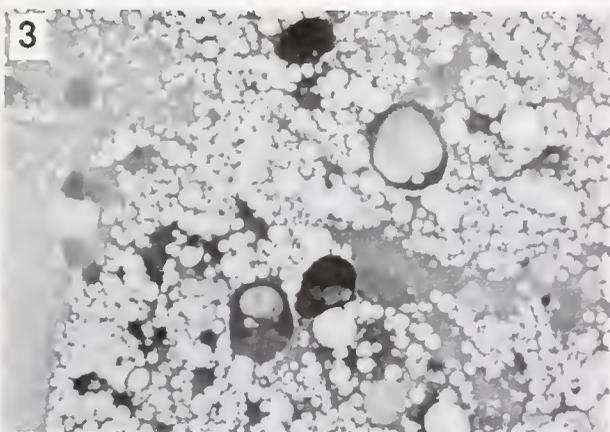


Figure 3. Ciliates in the stained hepatopancreas smear from #2 dead lobster (97 mm male) submitted April 13, 1990 from the Southport Island, Maine impounding facility showing more degenerated forms present than in the hepatopancreas of the recently dead lobster submitted on April 9, 1990. Included in this photo is an oval shaped ciliate that was apparently alive when the smear was prepared, two ciliates containing vacuolated areas and a rounded, swollen, degenerated ciliate. Hyman (1940) stated that protozoa tend to become highly vacuolated when aging, dying, starving, or under other adverse conditions.

#### Small Point, Maine

On May 23, 1990 a Small Point, Maine pound reported mortalities of lobsters that had been stocked prior to December, 1989. From the final 38 crates (approximately 3800 pounds) of lobsters removed from the pound, which had been floating for one week, 600 pounds were received dead following transport. Stained hemolymph slides from seven lobsters were negative for gaffkemia but numerous ciliates were present in the hemolymph of five lobsters, a moderate number of ciliates were present in one lobster, while the remaining lobster was negative for ciliates. Live ciliates were readily detectable in these infections by removing a section of gill or swimmeret and examining the vascular spaces within these structures at 100 $\times$  (Fig. 5). Bang (1983) stated that the ability of *Anophrys* (now *Mugardia*) to bend, twist, and push through narrow spaces is an adaptation to internal parasitism. Hepatopancreas smears were not prepared from these lobsters.

#### Transmission Experiments

Two experiments were conducted, one commencing April 13, 1990, the second May 8, 1990. In both instances, when hemolymph from ciliate-infected lobsters was injected into uninfected lobsters, the lobsters became positive for ciliate disease and the disease progressed in severity to death of the lobsters.

In the first experiment 0.5 ml of hemolymph from #2 lobster (97 mm male) of April 13, 1990, submitted from the Southport Island pound and containing numerous ciliates, was injected into an 85 mm male (#1 recipient) and into an 84 mm male (#2 recipient). Both recipients were obtained from DMR lobster project's holding tanks and tested negative for ciliates and gaffkemia. Subsequent hemolymph samples revealed a progressive increase in the number of ciliates in both recipients, #1 recipient died at 25 days post-injection, #2 recipient died 33 days post-injection (Table 2). Ciliate-infected lobsters became lethargic and weak before death. At the time of death, both recipients contained numerous ciliates in fresh hemolymph, stained hemolymph and stained hepatopancreas preparations. Recipient #1 had several small nodular growths on the intestinal wall. Giemsa-stained squashes of these growths revealed that the nodules contained tightly packed masses of degenerated ciliates. Ciliates in these masses appeared agglutinated and many ciliates had lost their individual identities (Fig. 6).

In the second experiment, Table 3A, on May 8, 1990 #1 recipient (83 mm male) received 0.2 ml of ciliate-infected hemolymph, while #2 recipient (89 mm female) received 0.4 ml of ciliate-infected hemolymph. The donor was the #1 recipient (85 mm male) of the first experiment that had died from ciliate disease on May 8, 1990. The recipients were again lobsters from the lobster project's holding tanks that tested negative for ciliates and gaffkemia. Hemolymph samples taken at intervals in this second experiment revealed a progressive increase in the number of ciliates in both recipient lobsters, but apparent differences in infection rates existed between the two. On day 27 post-injection #1 recipient, which had received 0.2 ml of ciliate-infected hemolymph, had only a few ciliates present, while #2 recipient, which had received 0.4 ml of ciliate-infected blood had hemolymph which was swarming with ciliates. Recipient #2 died 30 days post-injection and had numerous ciliates in its fresh and stained hemolymph and stained hepatopancreas preparations at the time of death. Recipient #1, on days 37 and 58 post-injection, showed

TABLE 1.

Occurrence of the parasitic ciliate *Mugardia*, (formerly *Anophrys-Paranophrys*), in lobsters, *Homarus americanus*, from a Southport Island, Maine impounding facility.

Date	Number of Lobsters Received and Condition	Carapace Length and Sex	Gaffkemia		Ciliates		Live Ciliates in Fresh Hemolymph
			Stained Hemolymph	Stained Hepatopancreas	Stained Hemolymph	Stained Hepatopancreas	
April 9, 1990	1 (dead)	88 mm female	neg	neg	numerous	numerous	not examined
April 11, 1990	#1 (live)	90 mm male	neg	neg	few	not done	moderate
	2 (live)	88 mm male	neg	neg	few	not done	moderate
	3 (moribund)	93 mm female	neg	neg	numerous	not done	numerous (blood milky white in color)
	4 (dead)	90 mm male	neg	neg	moderate	not done	moderate
	5 (dead)	95 mm female	neg	neg	numerous, many ciliates lysed, rod-shaped bacteria present	not done	numerous
April 12, 1990	#1 (live)	83 mm female	neg	neg	neg	not done	negative
	2 (live)	102 mm male	neg	neg	neg	not done	negative
April 13, 1990	#1 (dead)	89 mm female	neg	neg	neg	not done	negative
	2 (dead)	97 mm male	neg	neg	moderate	moderate	numerous
	3 (dead)	84 mm female	neg	neg	moderate	not done	numerous
	4 (dead)	90 mm female	neg	neg	numerous	not done	numerous
May 10, 1990	#1 (dead)	88 mm -	neg	neg	numerous	not done	numerous (blood milky white in color)
	2 (live)	20 mm -	neg	neg	neg	not done	negative
	3 (live)	31 mm -	neg	neg	neg	not done	negative

numerous ciliates in its fresh and stained hemolymph preparations. This lobster died sometime between 59–62 days post-injection; a post-mortem hepatopancreas smear was not prepared from this lobster.

In addition, on May 9, 1990 two lobsters, an 87 mm male and

an 89 mm male, obtained from the lobster project that tested negative for ciliates and gaffkemia were used as negative controls. One of these negative controls (89 mm male) was used as a donor of 0.2 ml of hemolymph to the other negative control (87 mm male). These lobsters were placed in the tank with the above two recipient lobsters and #2 recipient of April 13, which remained alive until May 16. Both negative controls of May 9, 1990 were still negative for ciliates and gaffkemia on the last bleeding date.

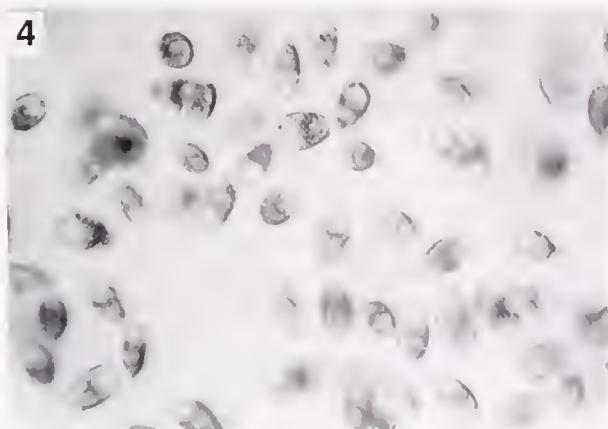


Figure 4. Numerous live ciliates of various sizes and shapes in the hemolymph of #2 dead lobster (97 mm male) submitted April 13, 1990 from the Southport Island, Maine impounding facility. A posterior contractile vacuole is noticeable, but cilia, while readily seen through the microscope, are not readily apparent in the photomicrograph. Note absence of hemocytes.



Figure 5. Photomicrograph of live ciliates in the vascular spaces within a gill of a 92 mm female lobster submitted on May 23, 1990 from a Small Point, Maine impounding facility.

TABLE 2.

Transmission Experiment #1. 0.5 ml of ciliate-infected hemolymph from the same donor was injected into the pericardial sinuses of two uninfected lobsters.

Donor: 97 mm male submitted April 13, 1990 from a Southport Island, Maine impounding facility.

Date (1990)	No. of Days Post-Injection (Apr. 13, 90) (water temp. 3°C)	Water Temperature (°C)	#1 Recipient, 85 mm Male, Received 0.5 ml of Ciliate-Infected Hemolymph.	#2 Recipient, 84 mm Male, Received 0.5 ml of Ciliate-Infected Hemolymph.
April 23	10	5	negative	1 ciliate
April 26	13	—	(no sample)	1 ciliate
May 7	24	7	Moderate number of ciliates in fresh and stained hemolymph preparations.	Moderate number of ciliates in fresh and stained hemolymph preparations. Few hemocytes present.
May 8	25	7	Lobster died. Numerous ciliates in fresh hemolymph, stained hemolymph and stained hepatopancreas preparations. Reduced numbers of hemocytes from normal.	(no sample)
May 16	33	8		Lobster died. Numerous ciliates in fresh hemolymph, stained hemolymph and stained hepatopancreas preparations. Near absence of hemocytes.

July 24, 1990 (day 76); these lobsters did not become infected by being in the same tank with the infected lobsters (Table 3B).

On May 10, 1990 two small lobsters received from the Southport pound, 31 mm and 20 mm, that tested negative for gaffkemia and ciliates were placed in the experimental tank. These lobsters remained negative throughout their captivity. The 20 mm lobster was last bled on July 5, 1990 and the 31 mm on July 24, 1990.

#### Size of Live Ciliates

Ciliate size can be quite variable within the same hemolymph sample. Typical sizes of live ciliates in the fresh hemolymph of #3

lobster of April 11, 1990 from the Southport pound were  $24.5 \times 17.5\mu\text{m}$ ,  $30 \times 21\mu\text{m}$ ,  $33.5 \times 25\mu\text{m}$  and  $35 \times 27.5\mu\text{m}$ . Recipient #1 lobster (85 mm male) from our first transmission experiment, on May 7, 1990 showed typical ciliate sizes of  $25 \times 16\mu\text{m}$ ,  $31 \times 20\mu\text{m}$ ,  $35 \times 20\mu\text{m}$ ,  $35 \times 24.5\mu\text{m}$  and  $40 \times 25\mu\text{m}$ . Recipient #2 lobster (84 mm male) on May 7, 1990 had typical ciliate sizes of  $30 \times 18\mu\text{m}$ ,  $30 \times 20\mu\text{m}$ ,  $35 \times 21\mu\text{m}$ ,  $35 \times 24.5\mu\text{m}$  and  $40 \times 20\mu\text{m}$ . Hemocytes measured  $12.5 \times 12.5\mu\text{m}$  and  $15 \times 15\mu\text{m}$ .

Variability in ciliate sizes was especially evident in the case of the 83 mm male (#1 recipient) being monitored in our second transmission experiment. On June 14, day 37 post-injection, at 12.8°C water temperature, numerous ciliates were present, showing the normal variability in ciliate sizes in the fresh hemolymph. However, on July 5, day 58, 13.9°C water temperature, a much greater variability in ciliate sizes was evident. In addition to the numerous ciliates present, larger adult forms and a greatly increased number of very small, young ciliates was evident, apparently having resulted from binary fission of adult forms between June 14 and July 5 (Fig. 7). Accompanying this increase in young ciliates was an increase in the number of hemocytes of the lobster. Typical measurements of these young ciliates in fresh hemolymph was  $20\mu\text{m} \times 20\mu\text{m}$  while other ciliates typically ranged from  $32\mu\text{m} \times 20\mu\text{m}$  to  $42\mu\text{m} \times 22\mu\text{m}$  with an atypically large ciliate of  $70\mu\text{m} \times 30\mu\text{m}$ . The lobster died sometime between July 6–9.

#### Ciliate Survival

Ciliates were still alive in hemolymph removed from the crusher and shredder claws of a recently dead 85 mm male lobster (#1 recipient of April 13, transmission experiment #1), after it had been allowed to reach room temperature (21°C).

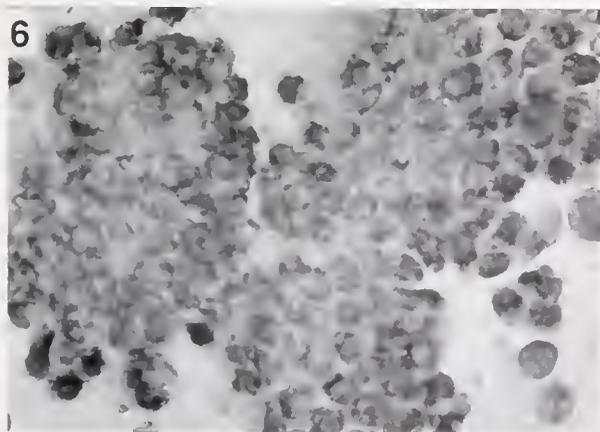


Figure 6. Giemsa-stained squash of a nodular growth on the external wall of the intestine of #1 recipient (85 mm male) in transmission experiment #1, 25 days post-injection, on May 8, 1990 showing tightly packed masses of degenerated ciliates.

TABLE 3A.

Transmission Experiment #2. 0.2 ml and 0.4 ml of ciliate-infected hemolymph from the same donor were injected, respectively, into the pericardial sinuses of two uninfected lobsters.

Donor: #1 recipient (85 mm male) of #1 experiment.

Date (1990)	No. of Days Post-Injection (May 8, 1990) (water temp. 7°C)	Water Temperature (°C)	#1 Recipient, 83 mm Male, Received 0.2 ml of Ciliate-Infected Hemolymph.	#2 Recipient, 89 mm Female, Received 0.4 ml of Ciliate-Infected Hemolymph.
May 21	13	8.9	negative	Several ciliates in fresh hemolymph
June 4	27	11.1	Several live ciliates	Hemolymph swarming with ciliates
June 7	30	10.5	(no sample)	Lobster died. Numerous ciliates in fresh hemolymph, stained hemolymph and stained hepatopancreas preparations. Few hemocytes present.
June 14	37	12.8	Numerous ciliates in fresh and stained hemolymph preparations. few-moderate hemocytes present.	
July 5	58	13.9	Numerous ciliates in fresh and stained hemolymph preparations. Small ciliates present, apparently from recent binary fission of larger ciliates. Moderate number of hemocytes.	
July 6-9	59-62		Lobster died	

Another recently dead lobster (84 mm male, #2 recipient of April 13) was kept refrigerated at 4.4°C and hemolymph samples were obtained from the abdomen 24 and 48 hours later. Ciliates were still alive after 24 hours and barely alive 48 hours later.

#### *Examination of Lobster Hemolymph and Hepatopancreas Slides from Past Years for Evidence of Ciliates*

Review of hemolymph and hepatopancreas preparations from lobsters submitted from Beals Island, Maine, February 11, 1980; Belfast, Maine, September 8, 1980; Kittery, Maine, July 16, 1986; Boothbay, Maine, November 3, 1987 and Bucks Harbor, Maine, December 3, 1987 from pounds that were experiencing mortalities showed that ciliates were not evident in hemolymph slides from 22 lobsters but were present in hepatopancreas pre-

parations from 13 of 16 of these lobsters. Thus, if only hemolymph slides had been prepared for evidence of gaffkemia, the fact that most lobsters contained hepatopancreas ciliates would not have been detected.

There were cases in these past collections where ciliates were either abundant enough to lead us to believe that ciliates may have contributed to mortalities, as in the Belfast sample, or prevalent enough, as in the Boothbay and Bucks Harbor samples, that other lobsters in the pounds were likely infected, perhaps more severely than those submitted for examination. The first case was from the Belfast sample (8 lobsters) where the only hepatopancreas slide that we have on hand showed numerous ciliates (4,270), plus heavy gaffkemia (Fig. 8). Ciliates were not detected in the hemolymph slide of this lobster, but, as in the hepatopancreas, heavy gaffkemia was present. Ciliates were not present in the he-

TABLE 3B.

Transmission Experiment #2—Controls (held in the same tank as #1 and #2 recipients and #2 recipient of the first transmission experiment).

Date	No. of Days Post-Injection (May 9, 1990) (water temp 7°C)	Water Temperature (°C)	#1 Control, 89 mm Male, Negative for Ciliates	#2 Control, 87 mm Male, Negative for Ciliates, Received 0.2 ml hemolymph From #1 Control on May 9, 1990
May 21	12	8.9°C	neg	neg
June 4	26	11.1°C	neg	neg
June 14	36	12.8°C	neg	neg
July 5	57	13.9°C	neg	neg
July 24	76	16.7°C	neg	neg

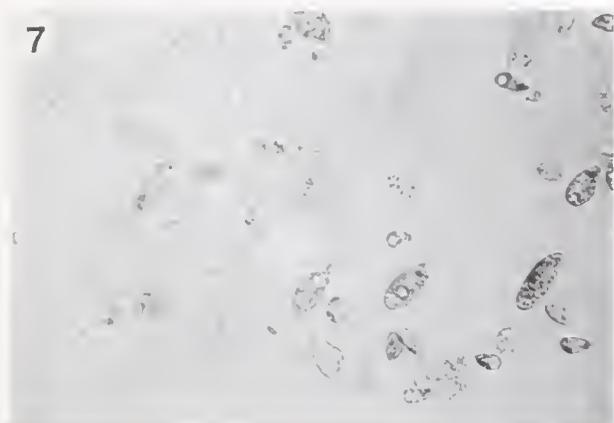


Figure 7. Photomicrograph of live ciliates in the hemolymph of #1 recipient (83 mm male) on July 5, 1990 (day 58 post-injection) in the second transmission experiment. In addition to the numerous ciliates present, larger adult forms and a greatly increased number of very small, young ciliates are evident, apparently having resulted from binary fission of adult forms since the last bleeding on June 14. Accompanying this increase in young ciliates was an increase in the number of hemocytes of the lobster. The seawater temperature from which this lobster was taken was 13.9°C (57°F).

molymph of seven other Belfast lobsters but heavy gaffkemia infections were present in the hemolymph of four of these seven lobsters. Another case was from the Boothbay sample (5 lobsters) where a hepatopancreas slide showed 1,150 ciliates present (Fig. 9) but no gaffkemia; the hemolymph slide was negative for ciliates and gaffkemia. Four other lobsters in the Boothbay sample also had hepatopancreas ciliates, ranging from 42 to 311 ciliates on the slides; there was no evidence of gaffkemia in the hemolymph or hepatopancreas slides. In the Bucks Harbor sample (6 lobsters), ciliates were evident in hepatopancreas slides from five of six lobsters, ranging from 65 to 1600 ciliates per slide. All six lobsters were negative for hepatopancreas gaffkemia and no gaffkemia or ciliates were evident in the only lobster from which hemolymph was drawn.

In the Beals Island sample (6 lobsters), hepatopancreas slides from two of three lobsters had ciliates, one slide had 143 ciliates,

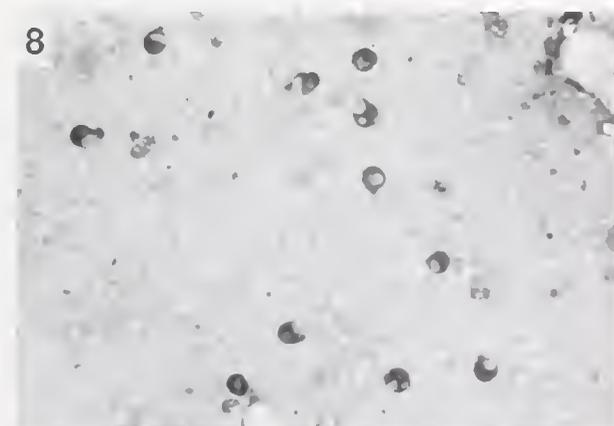


Figure 8. Pyriform to rod-shaped ciliates in the stained hepatopancreas smear from a lobster submitted from a Belfast, Maine pound on September 8, 1980. At least 4,270 ciliates were present on the slide.

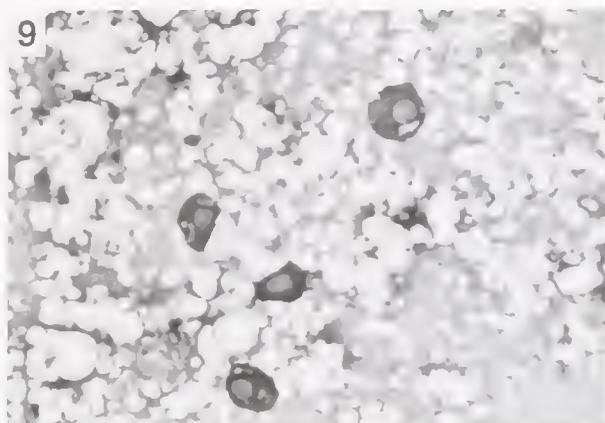


Figure 9. Ciliates in the stained hepatopancreas smear from a lobster submitted from a Boothbay, Maine impounding facility on November 3, 1987.

the other 322. Ciliates were not evident in hemolymph from these three lobsters from which hepatopancreas slides were prepared or in hemolymph from two other lobsters. Gaffkemia was not detected in hemolymph from five lobsters or in hepatopancreas preparations from three lobsters. In the Kittery sample ciliates were not evident in the hemolymph from the three lobsters examined or in the only hepatopancreas preparation that we have on file. Gaffkemia was not detected in the hemolymph or hepatopancreas from one lobster, one other lobster had light gaffkemia in the hemolymph, while the remaining lobster had heavy gaffkemia in the hemolymph.

*Examination of Fresh Unstained Hemolymph, Giemsa-stained Hemolymph and Giemsa-stained Hepatopancreas Smears from Freshly-Caught Lobsters from Ten Locations Along the Maine Coast and One Offshore Area for Evidence of Gaffkemia and Ciliates*

#### Gaffkemia

Gaffkemia was not evident in fresh unstained hemolymph or in stained hepatopancreas preparations from the 89 freshly-caught lobsters sampled, or in stained hemolymph smears that were prepared from 61 of these lobsters.

#### Ciliates in Fresh Unstained Hemolymph

Ciliates were present in the fresh hemolymph of one of 89 freshly-caught lobsters- in an 88.1 mm female from Pemaquid examined on November 30, 1990. This lobster, trapped on November 29, was dead-on-arrival at the DMR laboratory and its hemolymph contained numerous live ciliates and few hemocytes.

#### Ciliates in Stained Hemolymph

Ciliates were present in the stained hemolymph of two of 61 freshly-caught lobsters. Both lobsters were from a Pemaquid sample of seven received on November 30, 1990. The first lobster was the aforementioned 88.1 mm female which also showed numerous

ciliates in the stained hemolymph (Fig. 10) and the second was a 83.4 mm female with just a few (38) ciliates in the stained hemolymph—this lobster was also dead-on-arrival at the lab and had few hemocytes remaining.

#### Ciliates in Stained Hepatopancreas

Ciliates were present in various concentrations in the stained hepatopancreas smears of all 89 lobsters sampled, from nearshore to 75 miles offshore in 117 fathoms at Jordan Basin. Thus, ciliates can be present in the hepatopancreas when they are not detected in fresh or stained hemolymph preparations.

As in lobsters previously examined, stained hepatopancreas smears contained ciliates that were apparently alive when the smear was made and others that were apparently dead when the smear was prepared. The "live" ciliates were generally well-formed, i.e. elongate to pyriform in shape, stained a deep blue color, and the macronucleus and contractile vacuole were usually evident; "dead" ciliates had a swollen appearance, did not stain well and showed processes of degeneration (Figs. 11, 12).

Overall, the average number of ciliates in these hepatopancreas smears was quite variable, ranging from 0.02 ciliates to 56.0 ciliates per low power (100 $\times$ ) field per lobster (50 fields/lobster). Considering that there are approximately 360 low power (100 $\times$ ) fields in a typical hepatopancreas smear, there would be from 7.2 to 20,160 ciliates on a microscope slide made from a very small piece of tissue; the number of these microscopic ciliates present in the total mass of a lobster hepatopancreas can be tremendous.

There were substantial differences between geographic areas and between individual lobsters in the number of ciliates present in the hepatopancreas smears. Of the 11 areas sampled, Stonington lobsters had the lowest average number of ciliates/100 $\times$  field per lobster with  $0.5 \pm 0.35$  and the least variability in the range of ciliates/100 $\times$  field per lobster with 0.02–1.1, while Pemaquid lobsters had the highest average number and the most variability with  $12.9 \pm 19.6$  (0.4–56.0) ciliates/100 $\times$  field per lobster (Table 4). Pemaquid's high average was due to the two lobsters that were dead-on-arrival with averages of 14.4 and 56.0 ciliates/100 $\times$  field. Without these two lobsters, Pemaquid would have had a 3.9 average with a range of 0.4–9.1 ciliates/100 $\times$  field per lobster, which is within the values found for other areas. Second to Pemaquid, Scarboro lobsters had the highest average number of cil-

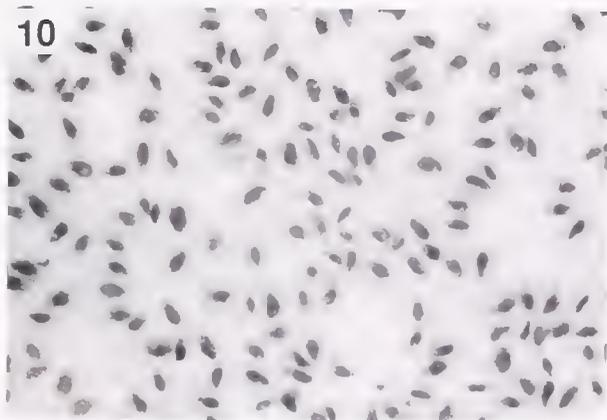


Figure 10. Numerous ciliates in the stained hemolymph smear of an 88.1 mm female lobster (#7) trapped off Pemaquid, Maine on November 29, 1990 that was dead-on-arrival at the DMR laboratory on November 30, 1990. Note rarity of hemocytes.

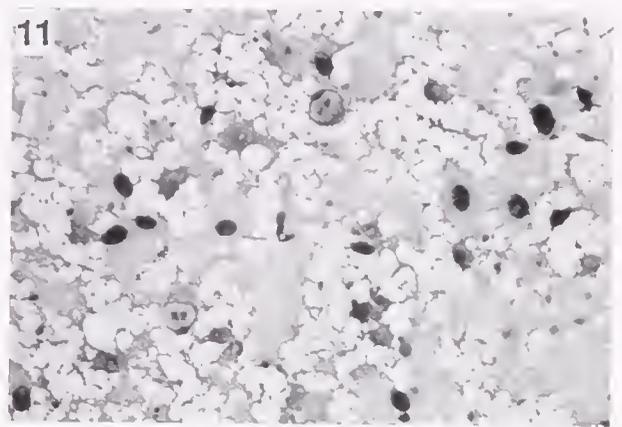


Figure 11. Ciliates in the stained hepatopancreas smear of the 88.1 mm female lobster (#7) trapped off Pemaquid, Maine on November 29, 1990 that was dead-on-arrival at the DMR laboratory on November 30, 1990. Note the swollen appearance of the degenerated ("dead") ciliates.

iates with 6.2/100 $\times$  field per lobster. Two lobsters in the Scarboro sample ranked third and fifth of 89 lobsters sampled in ciliate infection— a 91.6 mm female had 12.1 ciliates/100 $\times$  field and a 85.3 mm female had 12.6 ciliates/100 $\times$  field. The fourth largest concentration of ciliates occurred in a 103 mm male Jordan Basin lobster with 12.2 ciliates/100 $\times$  field.

Lobsters caught 9.0 miles offshore from Cutler in the channel near Seal Island and 75 miles offshore in 117 fathoms at Jordan Basin had as many ciliates in their hepatopancreas smears as nearshore lobsters (Fig. 13).

Larger (older) lobsters did not have a greater number of ciliates. For example, the smallest lobster of 89 sampled, from Cutler (in the channel near Seal Is.), an 81.6 mm female, had 5.9 ciliates/100 $\times$  field; the largest lobster sampled, from Jordan Basin, a 124.8 mm female, had 0.3 ciliates/100 $\times$  field.

The two most severely infected lobsters from Pemaquid had a much higher ratio of live to dead ciliates in their hepatopancreas smears than other lobsters in the Pemaquid sample and all other freshly-caught lobsters, which might be expected if the ciliates are proliferating and overtaking the lobster's system. In the recently

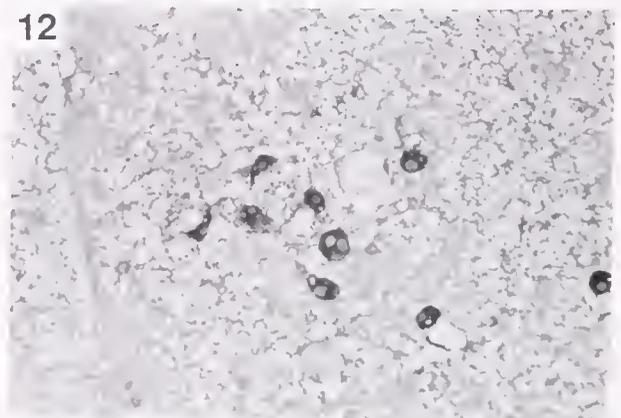


Figure 12. Ciliates in the stained hepatopancreas smear of a 91.6 mm female, freshly-caught lobster from Scarboro, Maine on November 27, 1990 showing ciliates that were apparently alive and others that were apparently dead when the smear was prepared.

TABLE 4.

Occurrence of the parasitic ciliate *Mugardia*, (formerly *Anophrys-Paranophrys*), in giemsa-stained hepatopancreas smears from freshly-caught Maine lobsters, *Homarus americanus*.

Location	Date Sampled	No. Lobsters Sampled	Range in Carapace Lengths of Lobsters Sampled	Mean, St'd Deviation and Range for the Number of Ciliates per 100x Field Per Lobster From Each Area (50 fields/lobster)
Scarboro	Nov. 27, 1990	7	85.3-93.6	6.2 ± 4.3 (2.2-12.6)
Phippsburg	Nov. 27, 1990	7	85.7-94.4	5.0 ± 2.4 (1.7-9.4)
Pemaquid	Nov. 30, 1990	7	82.7-89.1	12.9 ± 19.6 (0.4-56.0)
Tenants Hbr.	Nov. 20, 1990	7	83.1-88.9	2.9 ± 2.0 (0.5-6.1)
Stonington	Nov. 7, 1990	7	84.6-90.2	0.5 ± 0.35 (0.02-1.1)
Jonesport	Nov. 8, 1990	7	83.6-95.6	1.9 ± 1.2 (0.9-4.6)
South of Little Machias Bay	Nov. 30, 1990	7	82.6-89.6	2.9 ± 0.96 (1.7-4.7)
Cutler (in the channel near Machias Seal Island)	Nov. 29, 1990	7	81.6-88.6	5.5 ± 1.6 (2.1-6.9)
Cutler	Nov. 8, 1990	7	83.5-90.7	3.2 ± 1.6 (1.4-6.4)
Lubec-Trescott	Nov. 8, 1990	7	82.1-106.1	1.1 ± 1.1 (0.02-2.9)
Jordan Basin (NE portion-117 fathoms, 75 miles offshore, SSE of Jonesport)	Dec. 8, 1990	19	88.7-124.8	3.4 ± 3.5 (0.3-12.2)

dead Pemaquid 88.1 mm female, with 56 ciliates/100× field, of 2,779 ciliates counted, 86% were apparently alive and 14% apparently dead at the time the smear was fixed and stained. The other severely infected lobster (83.4 mm female) which was dead-on-arrival with 14.4 ciliates/100× field, of 720 ciliates counted, 78% were live and 22% degenerated. Five other lobsters in the Pemaquid sample that were alive when received had the opposite of the dead-on-arrival lobsters, with a higher percentage of dead ciliates than live in the hepatopancreas smears. For example, #1 (3.8 ciliates/100×), 222 ciliates counted, 14% live, 86% dead; #2 (1.2 ciliates/100×), 131 ciliates counted, 14% live, 86% dead; #3 (9.1 ciliates/100×), 521 ciliates counted, 28% live, 72% dead; #4 (0.4 ciliates/100×), 38 ciliates counted, 8% live, 92% dead and #6 (5.1 ciliates/100×), 257 ciliates counted, 21% live, 79% dead.

Lobsters from other locations showed the same general range of live to dead ciliates as in the above five lobsters from Pemaquid,

although several lobsters had a nearly equal number of live versus dead ciliates in their hepatopancreas smears.

For example, the Jonesport sample, where all lobsters were in good condition, showed a higher percentage of dead ciliates than live in six of seven lobsters, the remaining lobster (1.8 ciliates/100×), 250 ciliates counted, had 50% live and 50% dead ciliates. Ratios of live to dead ciliates in the other six lobsters were quite similar to each other: #1 (1.8 ciliates/100×), 362 ciliates counted, 28% live, 72% dead; #2 (0.9 ciliates/100×), 170 ciliates counted, 27% live, 73% dead; #4 (1.3 ciliates/100×), 186 ciliates counted, 24% live, 76% dead; #5 (1.6 ciliates/100×), 159 ciliates counted, 30% live, 70% dead; #6 (1.6 ciliates/100×), 307 ciliates counted, 21% live, 79% dead and #7 (4.6 ciliates/100×), 153 ciliates counted, 24% live, 76% dead.

#### Histopathology

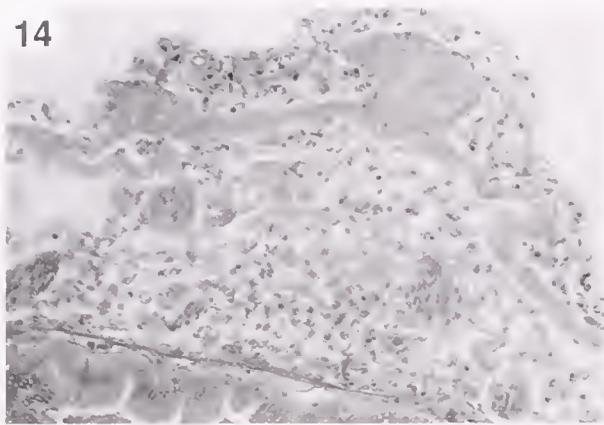
Examination of histological sections of intestine, hepatopancreas, heart and abdominal muscles prepared from two recently dead lobsters submitted from the Southport pound on April 13, 1990 showed that ciliates were responsible for destruction of tissue, especially in the intestinal walls of both lobsters.

Lobster #2 (97 mm male) which had numerous live ciliates in its hemolymph, contained numerous ciliates in the walls of the intestine accompanied by marked tissue destruction (Fig. 14). Ciliates contained what appeared to be small pieces of red staining muscle tissue. The hepatopancreas, overall, appeared to be in good condition, except for several small areas where tissue had been destroyed by ciliates and where ciliates were evidently in the process of consuming small groups of hemocytes (Fig. 15). The heart and the abdominal flexor and extensor muscles showed a few ciliates between the muscle bundles but without tissue involvement.

Lobster #4, (90 mm female) which had numerous live ciliates in its hemolymph, had evidently been dead long enough for autolysis of the tissues to occur, especially in the intestine and he-



Figure 13. Live and degenerated ciliates in the stained hepatopancreas smear of a 103 mm male lobster caught December 8, 1990, 75 miles offshore in 117 fathoms at the NE portion of Jordan Basin.



**Figure 14.** Histological section of the intestine of recently dead lobster #2 (97 mm male) submitted from the Southport Island, Maine impounding facility on April 13, 1990 showing numerous ciliates in the walls of the intestine accompanied by marked tissue destruction.

patopancreas. Numerous degenerated ciliates were evident in the wall of the intestine along with marked degeneration of muscle tissue. The entire hepatopancreas showed deterioration with tubules in some areas completely dissolved. There were several areas in the hepatopancreas where ciliates were associated with the degenerated tissue. The heart contained a moderate number of ciliates associated with necrosis of muscle tissue. The abdominal muscle showed a ciliate in the muscle fibers.

Histological sections were prepared from tissues removed from #1 recipient (85 mm male) of April 13, 1990 on the day of its death, May 8, 1990. Gross examination had revealed nodular growths on the intestinal wall which giemsa staining of a squash preparation showed to be filled with degenerated ciliates (Refer to Fig. 6). The histological section showed massive ciliate invasion of the intestinal wall with marked destruction of muscle tissue. Sections of several nodules revealed the interiors to be filled with ciliates in various stages of degeneration. The hepatopancreas showed only four ciliates, which did not appear viable, within the tissue. Three small areas in the green gland, situated in close proximity, contained round, necrotic unidentifiable bodies that



**Figure 15.** Histological section of the hepatopancreas of recently dead lobster #2 (97 mm male) submitted from the Southport Island, Maine impounding facility on April 13, 1990 showing destruction of tissue by ciliates.

were the size of ciliates. Ciliates were not evident in the heart tissue or esophagus, one ciliate was recognized in the stomach section and a few ciliates were evident in the gill section.

The ciliate infection in this lobster progressed very rapidly. On April 23 (10 days post-injection) the blood was negative for ciliates, 15 days later numerous ciliates were present in the blood and the lobster died. It is unknown whether the nodular growths on the intestinal wall occurred within this time frame or if the lobster had been successful in warding off ciliates by encapsulating them prior to our injecting it with ciliate-infected blood.

## DISCUSSION

The present study provides insight into the conjecture by Aiken et al. (1973) that "the crucial question is whether this ciliate has a long but unrecorded history of casual parasitism of *Homarus*, or whether it is in fact evolving into a major parasite of this species and will become a serious problem for lobster impounding facilities and aquaculture efforts." We have determined that this ciliate was present in lobster hepatopancreas but unrecognized as a potential pathogen in Maine lobsters dating back to 1980. In 1990, this ciliate was responsible for serious mortalities at two Maine impounding facilities, was present at various concentrations in the hepatopancreas of all wild lobsters examined, and was responsible for the deaths of two freshly-caught, market-size lobsters from a sample of seven submitted from the commercial fishery off Pemaquid, Maine.

The method of natural infection is unknown, but detailed gross examinations recorded for each lobster revealed that there were a number of portals of entry for pathogens in nearly every lobster, primarily from damaged antennae and walking legs, but also from damaged uropods and puncture wounds that had yet to heal.

The lobsters in the Southport impounding facility, which experienced severe mortalities in April, 1990, had been purchased from September to November, 1989, within a 50 mile radius of the pound. The pound owner thought he may have purchased a poor lot of lobsters from a particular area and thus introduced the disease. Another contemplated source was from a resident population of crabs in the pound area. We now know, from our examination of wild lobsters from ten locations along the Maine coast and one offshore area, that all these lobsters could have been carrying ciliates, at least in their hepatopancreas, when they were pounded and some may possibly have had ciliate infections in their hemolymph. Lobsters in the worst condition characteristically have had ciliates in their hemolymph, as well as their hepatopancreas.

Preliminary evidence suggests that there may be a relationship between the number of hepatopancreas ciliates and the point at which ciliates are found in the hemolymph. The two freshly caught Pemaquid lobsters, that were received dead, and that had the greatest number of hepatopancreas ciliates of 89 freshly-caught lobsters, at 14.4 and 56.0 ciliates/100 $\times$  field, also had hemolymph ciliates, while other wild lobsters and those reviewed from past collections, with hepatopancreas ciliates below 14.4 ciliates/100 $\times$  field, did not have ciliates in their hemolymph smears.

Stewart (1980) stated that the lobster has a comprehensive set of intrinsic defense mechanisms that include a bactericidal system, an agglutinin and opsonin system, and phagocytosis, 'all of which—when operating jointly—will effectively and quite specifically clear most foreign objects from the system.' Specifically, the

hepatopancreas contains a wide variety of enzymes that can be presumed to be active in the digestion of bacteria and exhibits bactericidal activity at a concentration five times greater per unit of tissue than in the circulatory system. It is also presumed that complete elimination of foreign agents eventually occurs in the hepatopancreas.

Healthy, feral lobsters in this study had a greater proportion of degenerated or dead ciliates in their hepatopancreas smears than the two wild lobsters which had recently died of massive ciliate invasion of the hemolymph, suggesting that the liver may act as a defense system against these parasites as well as bacteria. And, perhaps, as in the case of *Aerococcus viridans* var. *homari* (gaffkemia) disease, the lobster has not developed adequate defenses of resistance to the organism once the ciliates enter the hemolymph. That the lobster has some defense against these ciliates is evidenced by the finding of small nodular growths filled with degenerated ciliates on the intestinal wall of our #1 recipient (85 mm male) in our first transmission experiment.

Aiken et al. (1973) reported that parasitism by this ciliate occurred in water temperatures ranging from 0–10°C, appearing in November and disappearing the following March or April in two instances. Lobsters used for transmission experiments in the present study were held in a flow-through tank at West Boothbay Harbor, Maine from April to July, 1990. Ciliates were observed in lobsters taken from water temperatures ranging from 5°C in April to 13.9°C in July.

Something that should be considered in the transmission experiments of captive lobsters, where ciliate-infected hemolymph is injected into negative recipients, is that an unknown number of ciliates might already have been present in the hepatopancreas of these recipients and how this might affect the results.

Aiken and Waddy (1986) stated that the natural incidence and prevalence of this disease in lobsters was unknown, but appears to be cyclic, having been found in wild stocks three times in 14 years. All their cases occurred during winter months at water temperatures of 0–5°C, but further elaboration was not made. Our samples of freshly-caught lobsters were taken from November 7 to December 8, 1990, limiting our knowledge of ciliate presence in wild lobster populations to these months and year. Sea water temperatures for the locations from which lobsters were sampled were not taken, however, the daily average sea bottom temperature (–25.0 ft. MLW) for Boothbay Hbr., Maine for the month of November, 1990 was 9.2°C, with a range from 7.6–10.9°C (Smith 1991).

Maine lobsters are perhaps more severely infected with ciliates now, to the point of causing mortalities, than in the past. We know from this study that mortalities were associated with finding numerous ciliates in the hemolymph. If this disease was as prevalent in the past, then it would seem that investigators who routinely examine lobster hemolymph for gaffkemia would have noticed the ciliates in numbers great enough to question the reason for their occurrence. Also, ciliates were not as numerous in the review of our own past collections as in the present study.

In addition to the two lobster impounding facilities in this study that experienced mortalities from ciliate disease in 1990, a lobster submitted in January, 1991 from a Boothbay Harbor lobster holding pound showed numerous ciliates in its hemolymph. Other lobsters submitted from this pound had died of severe gaffkemia infections.

Losses can be substantial from any one or a combination of

these diseases, as in the Southport pound, where reportedly \$40,000 of losses occurred. The Boothbay Harbor pound was emptied and lobsters marketed as soon as possible (at a loss) before the entire contents succumbed to disease.

Aiken et al. (1973) reported that this parasite had been found in large numbers inside the body cavity of dead lobster larvae, but may have achieved entry post-mortem. The present study did not include larval lobsters and only two juvenile lobsters were examined. Further examinations are warranted to determine the possible effect of ciliate disease on natural mortality and recruitment of these lobsters into the fishery.

Although gaffkemia (*Aerococcus viridans*) was not detected in giemsa-stained hemolymph and hepatopancreas preparations from feral lobsters in the present study, Vachon et al. (1981) confirmed that 6.7% (27/399) of the feral Maine lobsters which they sampled were infected with this bacteria.

The finding of a ciliate in the fresh hemolymph preparation of one of eight rock crabs, *Cancer irroratus*, trapped in the Southport pound suggests that these ciliates occur in the resident crab population. Further sampling is needed, not only of rock crabs but of green crabs, *Carcinus maenas*, to determine to what extent this occurs and to compare the ciliate burden in crabs living in pounds where ciliate infected lobsters are or have been found with those from populations at a distance from the pounds. This may tell us whether resident crabs are likely to infect newly pounded lobsters, perhaps from lobsters eating infected crabs or by ciliates being released into the pound environment following the death of the host crabs.

This ciliate disease may well affect the willingness of foreign countries to import live Maine lobsters until more is learned about the prevalence and significance of this disease. Maine officials are currently searching for new markets overseas, particularly in Asia, and are also trying to crack an Australian ban on importing live North American lobsters. Dr. Sarah Kahn, the Australian embassy's veterinary counsel, stated that in order to remove the ban, Australian authorities would have to analyze the possible dangers of diseases being spread to native lobsters from their North American counterparts (Associated Press 1991). The advent of this ciliate disease adds another dimension to the problem and should provide impetus for further studies to determine its potential impact on our commercial lobster fishery and lobster impounding facilities.

#### ACKNOWLEDGMENTS

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## REPRODUCTIVE CYCLE AND THE EFFECT OF TEMPERATURE ON OOGENESIS OF *PANDALUS BOREALIS* KRØYER, 1838

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**ABSTRACT** The reproductive cycle in periodically isolated populations of *Pandalus borealis* was determined from variations in the relative frequencies of adult females in different reproductive stages and the presence of larvae in the plankton [in Gullmarsfjorden on the Swedish west coast]. Spawning takes place in September–October and hatching commences in March–April. Based on estimates of mean durations of oogenesis made from the variations in frequency of female reproductive stages and mean ambient temperatures from four different deep water stagnation periods in the fjord, an inverse relationship between temperature and time for oogenesis was found. These results verify laboratory findings concerning the temperature effect on the duration of oogenesis of the deep water prawn from the Gulf of Maine.

**KEY WORDS:** shrimp, *Pandalus*, reproduction, temperature

### INTRODUCTION

Gullmarsfjorden on the Swedish west coast contains a small stock of *Pandalus borealis* Krøyer 1838 which, due to the combined effect of a stratified water mass and bottom topography, becomes isolated annually from the neighboring large Skagerrak population. Most years the fjord population is strengthened through immigration during the water renewal period, but during deep water stagnation, shrimps are enclosed in the deep basin of the fjord which has stable hydrographical conditions [during each stagnation period] (Bergström 1991). This phenomenon, together with inter-annual variations in temperature, gives unique possibilities to test effects of temperature variations on the life cycle of this economically important, deep-water species.

Knowledge about the environmental factors governing hatching time and duration of oogenesis is important for stock management of shrimp and other crustaceans. Both factors ultimately affect the timing of larval release which, when considered in relation to the availability of planktonic food, may help explain differences in larval abundance and thus differences in year class strength (Stickney and Perkins 1981).

This paper describes the reproductive cycle of *P. borealis* in Gullmarsfjorden based on seasonal variations in relative frequency of female reproductive categories and occurrence of *P. borealis* larvae. Further, the relationship between temperature and the duration of oogenesis is investigated [based on estimates of population mean duration of oogenesis and mean ambient temperatures].

The only available information on this topic is reported by Stickney and Perkins (1980) who found an inverse logarithmic relationship between ambient temperature and the duration of oogenesis based on laboratory experiments which only covered approximately half of the period of oogenesis. The experiments included 72 female specimens equally distributed between four temperatures. The results indicated that the full term of oogenesis follows the equation  $\text{LOG}_{10} Y = 2.593 - 0.54 \text{LOG}_{10} X$ , where  $Y$  is the number of days from the first post-partem moult to the first egg extrusion and  $X$  is the temperature in °C. The assumption that oogenesis starts at the time of the first post-partem moult is based on results presented by Allen (1959) who showed that egg cell growth coincided with the first moult within ca. 1 month after hatching.

Worth noting in this context is the fact that the northern shrimp

is a stenoic, deep-living organism which does not readily lend itself to laboratory experimentation, a condition that is illustrated by a mortality of 10–28% during the 1–1.5 months that Stickney and Perkins (1980) kept *P. borealis* in the laboratory.

To test the validity of the model presented by Stickney and Perkins (1980) in the field, the enclosed fjord populations of *P. borealis* in Gullmarsfjorden were sampled during four deep-water stagnation periods (1984–1987).

General aspects of the reproductive cycle of *Pandalus borealis* throughout its discontinuous circumboreal distribution have been reviewed by Shumway et al. (1985). Female shrimp generally spawn once a year. Eggs are usually extruded in late summer to early autumn and are carried on the pleopods until spring. In the northern part of the shrimp's geographical range, however, deviations from this yearly pattern are common (Rasmussen 1953).

The reproductive cycle of *P. borealis* in the Gullmarsfjorden, Sweden has been briefly treated by Jägersten (1936), who reported mating and egg extrusion in September–October and hatching in the spring. Distribution and occurrence of the larvae of *P. borealis* have been studied in Kachemak Bay, Alaska, Sheepscot Bay, Maine, and in the Norwegian and Barents Sea (Shumway et al. 1985).

### MATERIALS AND METHODS

#### Study Area

The 120 meter deep Gullmarsfjorden is separated from the deeper parts of Skagerrak by a sill with an effective depth of about 40 meters. The water in the fjord is stratified, normally in three layers (Lindahl & Hernroth 1988). The two uppermost layers show, compared with the water below sill depth, a high degree of variation in salinity and temperature within each year. Below 50 meters of depth, during the 8–12 months following water renewal each year, the hydrographical conditions are very stable. The salinity of the deep water is relatively high (>34 0/00) and temperatures only vary between 4 to 7°C between different stagnation periods. The deep water in the fjord is normally exchanged each year (Svansson 1984, Lindahl and Perisinotto 1987, Lindahl and Hernroth 1988) in late winter or early spring.

A small stock of *P. borealis* found below sill depth seems to be isolated from the neighbouring Skagerrak population during most

periods of deep water stagnation. These temporally enclosed stocks can be viewed as well defined demes or populations. During periods of water renewal, however, immigration from the Skagerrak may be substantial. In three (1984, 1985, and 1987) out of four years studied, immigration of shrimp older than one year occurred during the deep water renewal (Bergström 1991).

#### Hydrography

Temperature and salinity were measured at 70, 90 and 110 m depths in the central parts of Gullmarsfjorden (Fig. 1) at least once a month throughout the study period. These parameters were measured with an accuracy of  $\pm 0.05$  degrees Celsius and  $\pm 0.05\%$  respectively, either with reversing thermometers and laboratory salinometer or with a CTD probe (Lindahl and Hernroth 1988, Bergström 1991). Temperature data taken during the stagnation periods were used to calculate mean temperatures and standard deviations representative of the four different stagnation periods in the deep basin of the fjord. Temperatures from the 70, 90 and 100 meter depths are shown in Figure 2. This figure also indicates the water renewals and the stability of the water temperature at these depths during deep water stagnation. Mean temperatures (Table 1) range from  $4.33 \pm 0.11$  to  $5.88 \pm 0.11$  during the four different stagnation periods.

#### Biological Sampling

Adult shrimp were collected by bottom trawling at approximately monthly intervals at three sites in the Gullmarsfjorden (Fig. 1) as described by Bergström (1991).

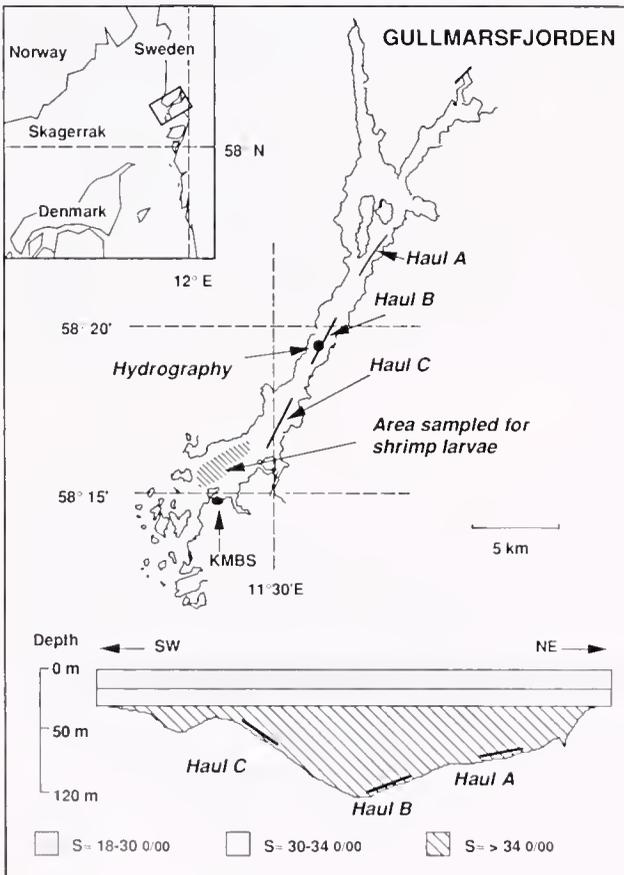


Figure 1. Map and depth profile over Gullmarsfjorden. The positions of the trawl hauls, the hydrographical station, and the area sampled for shrimp larvae are indicated in the figure.

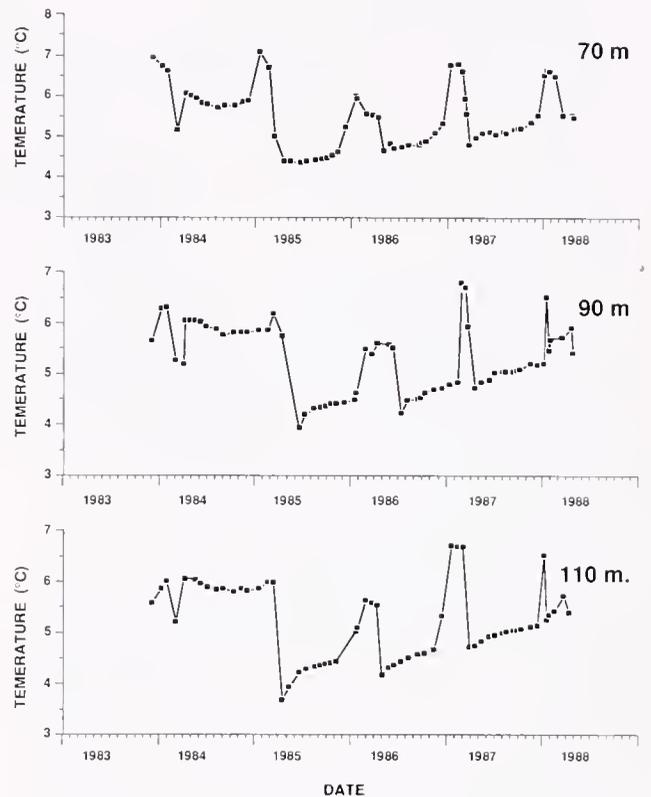


Figure 2. Temperature variations at 70, 90, and 110 meters of depth in Gullmarsfjorden during the studied period.

Information on abundance of shrimp larvae of different developmental stages was obtained from samples taken in the outer part of the fjord (Fig. 1) with a bongo net (mesh size 300 and 500  $\mu\text{m}$ ) fitted with a flow meter during late winter-early spring 1984–1987 (Table 2). Samples were taken approximately once a week. The aim of this sampling program was first to obtain information on the distribution and abundance of the ephyra larvae of *Aurelia aurita* (L.) (Gröndahl 1988) but since shrimp larvae were caught simultaneously the samples were analysed with respect to these also. This, together with the ice situation in the fjord, explains the lack of deeper hauls in the spring 1984–1986.

Sampling was begun after the ice cover of the outer part of the fjord had broken in 1984 and 1985. In 1986 two samples were obtained in February before the ice prevented sampling and sampling was resumed in early April. In 1987 no ice obstructed sampling. In 1984–1986 two oblique hauls, one to 20 meters of depth and another to 40 meters of depth were made at each sampling time. In 1987 an additional haul down to 60 meters was added to the sampling protocol.

#### Treatment of the Catch

Shrimp were mostly analyzed fresh, but occasionally the catch was frozen on board, and thawed for analysis upon return to the laboratory. Specimens were sexed on the basis of the shape of the endopodite of the first pleopod according to Allen (1959). The females were further sorted into four different categories according to their reproductive status (Rasmussen 1953):

1. Females without reproductive characteristics;
2. Females with ripe gonads visible through the carapace;
3. Berried females (females carrying eggs on the pleopods);
4. Females in breeding dress (long setae on the pleopods);

TABLE 1.  
Abundance of *P. borealis* larvae in Bongo-net catches, Gullmarsfjorden 1984–1987.

Depth Date	0–25 m			25–40 m			40–60 m					
	Abundance No/100 m <sup>3</sup>	No. in Stage			Abundance No/100 m <sup>3</sup>	No. in Stage			Abundance No/100 m <sup>3</sup>	No. in Stage		
		1	2	3		1	2	3		1	2	3
1984												
11 Apr	0	0	0	0	1.3	0	4	3		No sample		
16 Apr	0	0	0	0	1.8	0	2	12		No sample		
24 Apr	0.2	1	0	0	30.6	25	218	0		No sample		
8 May	0	0	0	0	0.1	1	0	0		No sample		
16 May	0	0	0	0	0.5	0	1	3		No sample		
28 May	0	0	0	0	0.2	0	2	0		No sample		
1985												
22 Mar	0.2	1	0	0	3.2	13	14	1		No sample		
29 Mar	0	0	0	0	0	0	0	0		No sample		
2 Apr	0.2	0	0	1	7.5	8	22	29		No sample		
16 Apr	0	0	0	0	0.4	3 stage 5				No sample		
1986												
3 Feb	0	0	0	0	0	0	0	0		No sample		
18 Feb	0	0	0	0		No sample				No sample		
2 Apr	2.9	15	5	4	4.7	25	14	2		No sample		
14 Apr	0.4	1	0	2	0.9	0	6	2		No sample		
22 Apr	0	0	0	0	0	0	0	0		No sample		
1987												
16 Feb	0	0	0	0		No sample				No sample		
23 Feb	0	0	0	0	0	0	0	0		No sample		
7 Mar	0	0	0	0		No sample				No sample		
12 Mar	0	0	0	0	0	0	0	0	6.5	12	3	0
19 Mar	1.1	3	1	0	0.3	1	0	0	4.9	11	4	0
20 Mar		No sample				No sample			1.1	2	1	0
26 Mar	0	0	0	0	0	0	0	0	0.3	0	0	1
31 Mar	0	0	0	0	0	0	0	0	1.3	6	2	0
22 Apr	0.2	0	0	1	0	0	0	0		No sample		
22 May	0	0	0	0	0	0	0	0	0	0	0	0

Females in the latter stage occur just after the hatching period and during spawning. The relative frequencies of these categories were determined in pooled catches of females from the three hauls taken during each sampling day. These frequencies were used to estimate mean duration of oogenesis (time span between the post-hatching moult and egg extrusion) in the population.

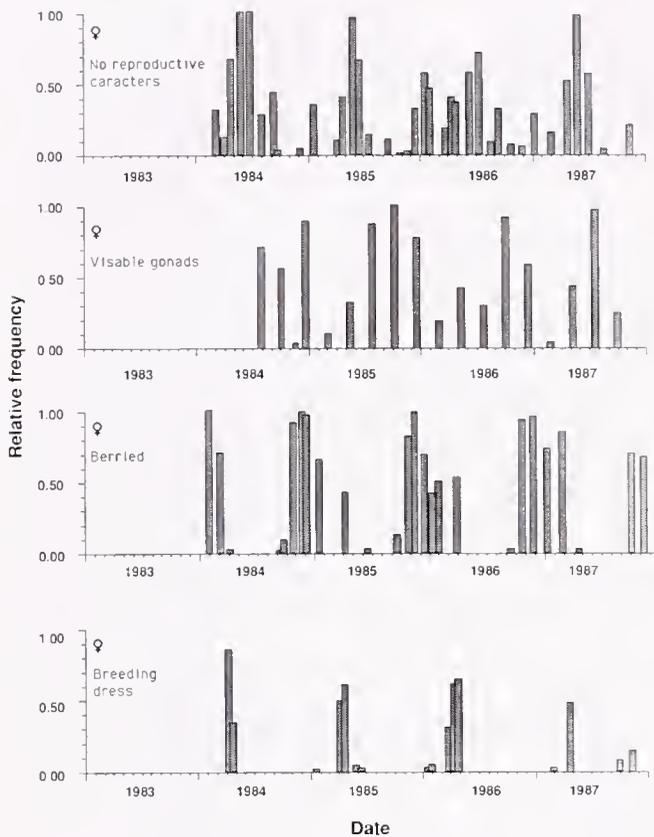
The date for the mean post-hatching moult (the date when  $\geq 50\%$  of the females have lost their breeding dress) was estimated by adding a period of 30 days to the estimated mean hatching date. Mean hatching date was assumed to be the mid-date between the

sample in which  $\geq 50\%$  of females were berried and the following sample with a  $\geq 50\%$  content of females still retaining their breeding dress (setae on pleopods). The more direct way of estimating the date for post-hatching moult by use of the frequencies of females in breeding dress and females showing no reproductive characteristics, was not used because sex change activity in the fjord population peaks during late winter-early spring. This sex change activity, together with the post-hatching moult, produces an increasing frequency of females which have no reproductive characteristics. This mixed origin of the females without reproductive characteristics makes direct estimation of the mean post-hatching moult very difficult, which motivates the use of the alternative method of adding 30 days to the hatching date. The addition of a 30 day period to the mean hatching date was deemed realistic based on information on hatching and moulting of *P. borealis* in the nearby Oslofjorden and Skagerrak given by Rasmussen (1953) and on data from the North Sea given by Allen (1959). In addition, the dates obtained by this calculation fit well with the observed variations in frequencies of females in breeding dress and females showing no external reproductive characteristics (Fig. 3) and the occurrence of shrimp larvae in Gullmarsfjorden (Table 1). The mean date for egg laying (end of oogenesis) was assumed to be the mid-date between the sample in which  $\geq 50\%$  of females had visible gonads and the closest following sample containing  $\geq 50\%$

TABLE 2.

Estimated population mean dates for female post-hatching moult and highest spawning activity in the Gullmarfjord population of *P. borealis* during stagnation periods 1984–1987.

Stagnation Year	Mean Post Hatching Moulting Date	Mean Spawning Date	Duration of Oogenesis	Mean Temp. $\pm$ s.d. (°C)
1984	19 Apr	17 Sep	151	5.88 $\pm$ 0.11
1985	2 Apr	5 Oct	183	4.33 $\pm$ 0.12
1986	4 Apr	1 Oct	180	4.56 $\pm$ 0.21
1987	30 Mar	10 Sep	164	5.04 $\pm$ 0.09



**Figure 3.** Relative frequencies of different female reproductive stages of northern shrimp, *P. borealis*, in samples taken in Gullmarsfjorden the years 1984–1987.

berried females. In the 1987 data this date was adjusted to a date 10 days earlier based on the occurrence of females in breeding dress (indicating spawning activity) in the earlier of the two sample. The time period in days between these estimated dates was calculated for each of the studied stagnation periods.

After extracting ephyrae from the bongo hauls, shrimp larvae were picked out under a magnifying glass. The shrimp larvae were classified according to their development stage (Berkely 1930) with the aid of a stereo microscope.

#### Statistics

The possible correlation between mean ambient temperature and the estimated durations of oogenesis each of the studied years was examined by correlation analyses. After correlation analysis, linear, logarithmic and exponential regressions describing the relationship between mean temperature and mean duration were calculated using the least square method (Sokal and Rohlf 1969).

## RESULTS

#### Female Reproductive Stages

Figure 3 illustrates the general reproductive cycle of female *P. borealis* in the fjord. In September, the relative frequencies of berried females increase and the proportion of females with ripe gonads decreases indicating the spawning period. Females carry eggs over the winter until hatching commences in March–April as shown by decreasing proportions of berried females and increasing proportions of females with remaining breeding dress (Fig. 3).

During the latter part of the same period, larvae in stages 1, 2, and 3 occurred in the bongo samples (Table 1). After losing the breeding dress through moulting in April–May, the female shrimp shows no reproductive characteristics during the early summer months (Fig. 3). A few females of this category were also observed in the winter and early spring catches, probably due to sex change activity in the population. The proportion of females with ripe gonads gradually increases [throughout the summer] until the spawning period starts in September–October (Fig. 3, Table 2).

#### Temperature and the Duration of Oogenesis

Estimates of the durations of oogenesis, which ranged from 151 to 183 days in the four studied stagnation periods, are presented in Table 1. There is a significant negative correlation ( $R = -0.98$ ,  $r^2 = 0.95$ ) between observed stagnation mean temperatures and estimated durations of oogenesis ( $p > 0.05$ ). Fitting of logarithmic,  $Y = 339.61 - 245.99 \cdot \text{LOG}_{10} X$  ( $r^2 = 0.960$ ), ( $Y = \text{duration in days}$ ,  $X = \text{average temp in } ^\circ\text{C}$ ), exponential  $Y = 337.19 \cdot 10^{(-46234e^{-2X})}$  ( $r^2 = 0.959$ ) and linear  $Y = 302.94 - 18.59 X$ , ( $r^2 = 0.952$ ) functions reveal that the logarithmic model gives the best fit to the empirical data. Figure 4 shows the estimated durations plotted versus mean temperatures and the fitted, logarithmic function.

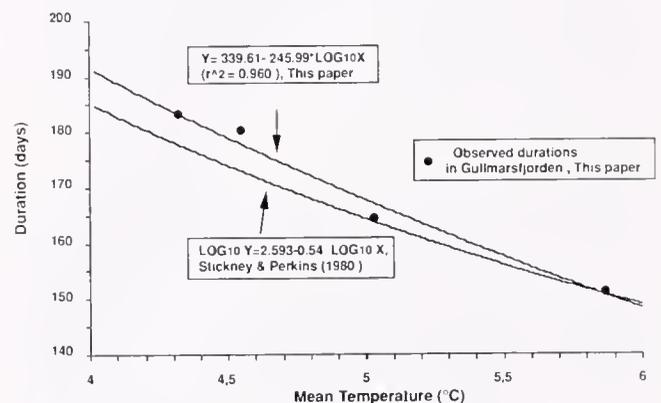
#### Larvae

Densities of larvae (Table 1) were low and variable in all analysed samples. Maximum abundance was found in late April 1984 with 31 larvae/100 m<sup>3</sup>. Early stage larvae (stages 1–3) were most abundant in March–April in the deeper hauls. Only in mid April 1985 a few stage 5 larvae were caught in a haul taken between 25 and 40 meters of depth.

## DISCUSSION

The frequencies of female reproductive stages and occurrence of larvae indicate that the period from spawning to hatching of *P. borealis* in Gullmarsfjorden is late September–early October to March–April. This duration of 6–7 months agrees with the general information about the shrimp population in Gullmarsfjorden given by Jägersten (1936) and with durations reported from southern Norway (Rasmussen 1953), the Gulf of Maine (Haynes and Wigley 1969) and the Gulf of Alaska and Kodiak area Shumway et al. (1985).

Larvae occur during the hatching time (March–April) in the



**Figure 4.** Plot showing logarithmic relationships between mean ambient temperature and the duration of oogenesis under laboratory conditions (Stickney and Perkins 1980) and in Gullmarsfjorden.

fjord and seem to be most abundant in the 25–60 meters depth interval. The observed peak densities in Gullmarsfjorden were generally lower than densities (800–300 larvae/1000 m<sup>3</sup>) reported from the Gulf of Maine 1977–1979 by Stickney and Perkins (1981). Only in April 1985 did the abundance of larvae reach a peak (306 larvae/1000 m<sup>3</sup>) comparable with that of the year having lowest abundance in the Gulf of Maine (1978); in the other years studied, abundances were 10–20 times lower in Gullmarsfjorden than in the Gulf of Maine. Despite these low densities of larvae, *P. borealis* is well established in the fjord, a fact that is illustrated by the presence of a small commercial shrimp fishery established in the beginning of the 20th century. With this background it seems possible that the immigration of adult shrimp during the water renewal period, which occurs most years (Bergström 1991) plays the most important role in maintaining the fjord stock. The observed variations in larval abundance between samples (Table 1) in Gullmarsfjorden were probably caused by the highly dynamic hydrographical pattern of the water layers above sill depth in the fjord. The topmost water layer has a maximum residence time of 12 days and the water just above sill depth has a maximum residence time of less than one month (Lindahl and Perisinotto 1987 and Lindahl and Hernroth 1988).

#### Temperature Effects on Oogenesis

The total duration of the reproductive cycle for female *P. borealis* can be viewed as a function of time for somatic growth, oogenesis and embryonic development. According to laboratory findings by Stickney and Perkins (1980) both the duration of oogenesis and the embryonic development are inversely related to ambient temperature. In the case of hatching time this can not be tested on the temporarily enclosed populations of shrimp in Gullmarsfjorden because of the recurrent but variable immigration of older females to the fjord during the water renewal periods each year. The effect of temperature on the duration of oogenesis can, however, be assessed by correlating population average durations of oogenesis with the average temperatures in deep part of the fjord basin during the four studied stagnation periods. Thus the

temporarily enclosed shrimp populations in the fjord offer a unique possibility to test the relationship between reproduction and temperature outside the laboratory. The results indicate a strong correlation between temperature and time for oogenesis and generally verifies the findings of Stickney and Perkins (1980). The relationship between temperature and time for oogenesis can be described with logarithmic functions, [in both the laboratory experiments and in the case of the stagnation populations from Gullmarsfjorden] although exponential and linear equations give almost as good fit to the data from Gullmarsfjorden within the investigated temperature interval. Stickney and Perkins (1980) gave the equation  $\text{LOG}_{10} Y = 2,593 - 0,54 \text{ LOG}_{10} X$ , where Y is the number of days until extrusion and X is the temperature in °C based on laboratory experiments. This model predicts a full term of oogenesis of 164 days at 5°C (Fig. 4). Based on the best fitting model ( $Y = 339,61 - 245,99 \text{ LOG}_{10} X$ ) from Gullmarsfjorden, oogenesis at 5°C takes 168 days. Considering the possible inaccuracies both in determining the duration of oogenesis from frequency data in trawl catches and from extrapolations from 1–1.5 month long experiments (Stickney and Perkins 1980) these results are surprisingly consistent.

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## POPULATION GENETIC DIFFERENTIATION OF THE PINK SHRIMP, *PANDALUS BOREALIS* KRØYER, 1838, FROM THE BARENTS AND BERING SEAS

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**ABSTRACT** Population differentiation of the pink shrimp (*Pandalus borealis*) was investigated at four allozyme loci (Gpi, Pgm, Mdh, Fdh) in samples from the Barents Sea, the Bering Sea and the Sea of Japan. Preliminary results for the Bering Sea and the Sea of Japan have been reported (Kartavtsev et al. 1991a, 1991b). There was statistically high, significant heterogeneity of allele frequencies among cohorts from different sea basins at all four loci. Within any sea, populations were homogeneous. Population genetic differentiation was measured by  $D_m$ ,  $D_{ST}$  and  $F_{ST}$ . The level of gene exchange ( $N_m$ ) between populations measured by  $F_{ST}$  is discussed.

**KEY WORDS:** pink shrimp, *Pandalus*, genetics, population structure.

### INTRODUCTION

The pink shrimp (*Pandalus borealis* Krøyer, 1838) is one of the most abundant species of the family Pandalidae. It is commercially harvested by otter trawl in the Pacific and Atlantic basins. One region of Spits Bergen in the Barents Sea provided a total catch of 128,000 metric tons per year in the middle 80-th alone (Bull. Stat. ICES 1988).

Biology of the pink shrimp has been reviewed by Shumway et al. 1985. The species has circumboreal distribution in the Northern Hemisphere but occurs in subarctic waters as well. Like others of the family, the pink shrimp is a protandric hermaphrodite: young individuals are males and change to females at the age of 3-8 years depending upon location. The planktonic stage exists for 3-4 months (Shumway et al. 1985), contributing to the high migratory capacity of the species.

For the pink shrimp, as for most commercially exploited species, definition of the number of reproductive units (populations) in an area, and evaluation of the degree of their genetic differentiation are important parameters. Until recently the population structure of the pink shrimp has been little studied, and then only by routine techniques of population biology and morphometrics. Past investigations were aimed only at shrimp from the Barents Sea (Kuznetsov 1964, Bryazgin 1970, Bryazgin and Rusanova 1974, Berenboim 1978, 1982, Teigsmark 1983, Lysyi 1988), and may reflect specific conditions at high latitudes. Our previous paper (Kartavtsev et al. 1991a) has established that: 1) the local shrimp collections within any given sea are genetically homogeneous and are apparently parts of one Mendelian population, and 2) the Sea of Japan and the Sea of Okhotsk on the one hand, and the Sea of Japan and the Bering Sea on the other hand, are inhabited by different populations possessing early differentiated gene pools. Our morphometric investigation has shown that shrimps obtained from the same sea are much more phenotypically homogeneous than those taken from different seas (Kartavtsev et al. 1991b). It is also of interest to ascertain how much the shrimps taken from the Atlantic, Pacific and Arctic oceans differ genotypically and phenotypically.

In the present paper we: 1) estimate the degree of genetic differentiation among shrimp in the Barents Sea; and 2) examine the relative allozyme divergence of pink shrimp inhabiting the Barents Sea from populations of the Bering Sea and the Sea of Japan.

### MATERIALS AND METHODS

Shrimp were obtained by bottom trawling from the research vessel (RV) "Captain Shaitanov" in the Barents Sea (BR) and RV "Professor Levanidov" in the Bering Sea (BS). Barents Sea samples were taken from the following locations:

Sample Sites	Locations	Depth (m)	Date
1. BR1	74°50'N/18°05'E	315	13.07.89;
2. BR2	75°35'N/16°45'E	215	27.09.89;
3. BR3	79°42'N/09°32'E	186	03.07.89;
4. BS5	60°38'N/178°57'W	270	18.07.89;
5. BS6	60°33'N/178°17'W	171	18.07.89.

Protein extraction was according to Korochkin, (1977). Enzymes were analyzed by starch gel electrophoresis (14-15% gel). Three different buffer systems were used with similar resolution: A—pH 8.1 tris-EDTA-borate buffer (TEB1, Korochkin et al. 1977); B—pH 7.4 tris-EDTA-maleate buffer (TEM, Shaw and Prasad 1970) and C—pH 8.6 tris-EDTA-borate buffer (TEB2, Korochkin 1977). Four enzymes were studied: 1—glucose phosphate isomerase (GPI, EC 5.3.1.9, locus Gpi), 2—phosphoglucosmutase (PGM, EC 2.7.5.1, Pgm), 3—malate dehydrogenase (MDH, EC 1.1.1.37, Mdh), 4—formaldehyde dehydrogenase (FDH, 1.2.1.1, Fdh). Under our experimental conditions, buffer A gave best resolution for GPI. For PGM and MDH buffer C gave the best resolution. The best results for FDH were obtained using buffer B. Stainings of GPI, PGM and MDH were performed as described by Shaw and Prasad (1970). FDH activity was measured as suggested by Balakirev and Zaykin (1990). Phenotype-genotype interpretations were conventional (Korochkin 1977).

Statistical analysis was performed on an EC-1060 computer

with the BMDP program package (Dixon 1977). Agreement between observed and expected genotype frequencies was tested with the standard chi-square test. Homogeneity of allele frequencies was estimated with different algorithms; all gave results similar to the generally used Workman-Niswander's chi-squares test (Workman and Niswander 1970). Cumulative differences in allelic frequencies at the loci were estimated by the unbiased minimal genetic distance  $D_m$  (Nei 1978). Genetic differentiation among populations of different size were measured by  $D_{ST}$  and  $F'_{ST}$  statistics (Nei 1987). The extent of differences between samples and the general level of differentiation were examined with the discriminant analysis techniques included in the BMDP package.

## RESULTS AND DISCUSSION

Several new rare alleles were observed. For statistical analysis corresponding heterozygote genotypes were united with the nearest ones from three common heterozygote genotype classes, as in our previous paper (Kartavtsev et al. 1991a).

Alleles were numbered from anode to cathode (Table 1). The genotype distributions do not differ among males, females and hermaphroditic individuals, thus it is possible to use the combined data set, including all specimens examined.

We report here new results on allele frequencies at four allozyme loci (Table 1). The average allele frequencies for the pop-

TABLE 1.

Allele frequencies at loci Gpi, Mdh, Pgm and Fdh in samples of the pink shrimp *Pandalus borealis* and results of chi-square test goodness of fit between observed and expected genotype frequencies.

Sample	N	Allele Frequencies ( $p \pm SE$ )			$H_o$	$\chi^2$
		$P_1$	$P_2$	$P_3$		
		Gpi				
JS	457	0.063 $\pm$ 0.008	0.934 $\pm$ 0.008	0.003 $\pm$ 0.002	0.1204	4.22
BS5	109		1.000		0.0000	
BS6	70		1.000		0.0000	
BS	469		0.998 $\pm$ 0.001	0.002 $\pm$ 0.001	0.0043	0.01
BR1	96		1.000		0.0000	
BR2	98		1.000		0.0000	
BR3	94		0.995 $\pm$ 0.005	0.005 $\pm$ 0.005	0.0106	0.01
BR	288		0.998 $\pm$ 0.002	0.002 $\pm$ 0.002	0.0035	0.01
		Pgm				
JS	452	0.018 $\pm$ 0.004	0.276 $\pm$ 0.015	0.706 $\pm$ 0.015	0.3761	7.44
BS5	109	0.028 $\pm$ 0.011	0.078 $\pm$ 0.017	0.894 $\pm$ 0.020	0.1839	0.88
BS6	70		0.079 $\pm$ 0.022	0.921 $\pm$ 0.022	0.1571	0.47
BS	482	0.023 $\pm$ 0.005	0.060 $\pm$ 0.008	0.917 $\pm$ 0.009	0.1473	—*
BR1	96		0.005 $\pm$ 0.005	0.995 $\pm$ 0.005	0.0104	0.00
BR2	98		0.010 $\pm$ 0.007	0.990 $\pm$ 0.007	0.0204	0.02
BR3	94			1.000	0.0000	
BR	288		0.005 $\pm$ 0.003	0.995 $\pm$ 0.003	0.0104	0.04
		Mdh				
JS	440	0.873 $\pm$ 0.011	0.127 $\pm$ 0.011		0.2090	1.53
BS5	109	0.541 $\pm$ 0.034	0.459 $\pm$ 0.034		0.5504	1.34
BS6	70	0.657 $\pm$ 0.040	0.343 $\pm$ 0.040		0.3429	4.02 <sup>+</sup>
BS	469	0.579 $\pm$ 0.016	0.421 $\pm$ 0.016		0.5096	0.96
BR1	92	0.522 $\pm$ 0.036	0.478 $\pm$ 0.036		0.5217	0.18
BR2	98	0.510 $\pm$ 0.036	0.490 $\pm$ 0.036		0.5102	0.04
BR3	94	0.516 $\pm$ 0.036	0.484 $\pm$ 0.036		0.6064	4.26 <sup>+</sup>
BR	284	0.516 $\pm$ 0.021	0.484 $\pm$ 0.021		0.5458	2.44
		Fdh				
JS	306	0.860 $\pm$ 0.014	0.057 $\pm$ 0.009	0.083 $\pm$ 0.011	0.2345	5.58
BS5	109	0.963 $\pm$ 0.013	0.023 $\pm$ 0.009	0.014 $\pm$ 0.008	0.0734	0.23
BS6	70	0.964 $\pm$ 0.016	0.021 $\pm$ 0.006	0.015 $\pm$ 0.010	0.0571	0.87
BS	457	0.980 $\pm$ 0.005	0.010 $\pm$ 0.003	0.010 $\pm$ 0.003	0.0372	9.08 <sup>+</sup>
BR1	61	0.975 $\pm$ 0.014	0.016 $\pm$ 0.011	0.009 $\pm$ 0.009	0.0492	0.06
BR2	98	0.974 $\pm$ 0.011	0.010 $\pm$ 0.007	0.016 $\pm$ 0.009	0.0510	0.08
BR3	94	1.000			0.0000	
BR	253	0.984 $\pm$ 0.006	0.008 $\pm$ 0.004	0.008 $\pm$ 0.004	0.0316	0.01

Note: dash indicates that values of chi-square were not calculated because of insufficient fullness in some frequency cells. N—the number of individuals examined;  $H_o$ —the observed frequency of heterozygotes in the sample; BS5–BS6—shrimp samples from the Bering Sea; BR1–BR3—those from the Barents Sea; JS, BS and BR—the pooled shrimp samples from the Sea of Japan, the Bering Sea and the Barents Sea (for BS—including 1988; Kartavtsev 1991a). Alleles were numbered from anode to cathode on the gels. Significance level:  $p < 0.005$ .

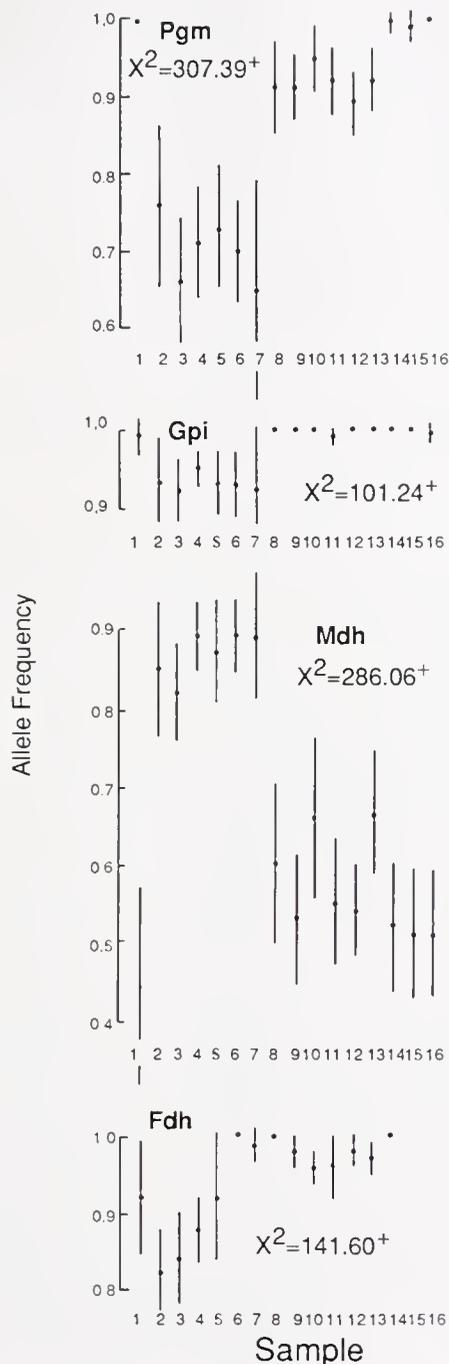


Figure 1. The frequency variability in common alleles at the Pgm, Gpi, Mdh, and Fdh loci in samples of the pink shrimps *Pandalus borealis*.

On the ordinate are the allele frequencies. The location of the sampling points are as follows: 1—Okhotsk Sea, 2–7—Sea of Japan (JS5, JS1, JS2, JS2', JS3, JS4), 8–13—Bering Sea (BS1–BS6), 14–16—Barents Sea (BR1–BR3). Dots indicate the frequency values, vertical bars the confidence intervals (95%). The frequency values for samples 1–11 are taken from (Kartavtsev et al. 1990a). The values of chi-square tests for heterogeneity computed from the whole sample set are listed in the bottom. The significance level for all samples— $p < 0.001$ .

ulations examined previously, from the Bering Sea and the Sea of Japan (Kartavtsev et al. 1991a), are included for comparison (these data represented by numerals for BS and JS, see Table 1). The average allele frequencies for samples from the Bering Sea include the new data obtained in 1989 (Table 1). A single sample from the Sea of Okhotsk was included when the total heterogeneity throughout the whole area was estimated, and it is also indicated on Fig. 1, but is not discussed further as it added no new information beyond that published previously (Kartavtsev et al. 1991a).

We have found Hardy-Weinberg equilibrium at most of the loci examined in the samples: the observed genotype frequencies agreed well with expected ones (Table 1). A small deficiency of heterozygotes was found in sample BS6 and an excess of heterozygotes in sample BR3 were found for locus Mdh; in the total distribution they compensated each other (Table 1).

The allele frequency variability had common features: within the same sea no significant differences were observed, whereas the samples from different seas differed strikingly (Fig. 1). The sample set of the pink shrimp from the Barents Sea is not notable among others for the allele frequency variability: we did not notice heterogeneity in the common alleles at any locus (Table 2).

When the samples collected in the eastern Bering Sea in 1989 (see Materials and Methods) were combined with four samples from the western Bering Sea collected in 1988 and already examined (Kartavtsev et al. 1991a), we again found no heterogeneity for the Bering Sea population at the four loci studied (Table 2). The corresponding cumulative value of chi-square in the test of heterogeneity for the four loci is:  $\chi^2_{BS} = 29.59$  ( $df = 20$ ,  $p < 0.1$ ).

Among separate loci the results of heterogeneity test in the sample set from the Barents Sea were the same as those for the Bering Sea (Table 2). The cumulative value of chi-square in the test of heterogeneity for all four loci (Gpi, Pgm, Mdh and Fdh) in the Barents Sea came to the:  $\chi^2_{BR} = 8.79$  ( $df = 8$ ,  $p < 0.5$ ) (Table 2). Thus, all populations of the pink shrimp from separate seas, including the sea of Japan which we examined earlier, were genetically homogeneous (Table 2). These results agree well with Hardy-Weinberg equilibria which were found both in the individual and total samples for the different seas (Table 1).

Samples taken from the same sea in different years do not differ in allelic frequencies at any of the four loci (Fig. 1, Table 1). For the Sea of Japan the samples obtained in 1987 do not differ from those of 1988 (Kartavtsev et al. 1991a; see also samples JS5 and JS1–JS4 in Fig. 1). As mentioned above, the homogeneity among samples in the Bering Sea remains throughout all data of 1988–1989. The absence of differences in the common allele frequencies exist at the Pgm locus in the sample collected in 1972 in the Bering Sea ( $P_3 = 0.924 \pm 0.009$ ; Johnson et al. 1974) and in our samples of 1988–1989— $\bar{p}_3 = 0.917 \pm 0.009$  (Table 1, BS). This suggests the possibility of the long-term stability of allele frequencies in the separate population units.

In striking contrast to the homogeneity within sea basins were comparisons of allele frequencies throughout the whole sample set or in paired groups of populations from different seas. The chi-square values in the test of heterogeneity calculated at a locus sequence Pgm (1), Gpi (2), Mdh (3) and Fdh (4) for the whole sample set were as follows (Table 2):  $\chi^2_{(1)} = 307.39$  ( $df = 15$ ,  $p < 0.001$ );  $\chi^2_{(2)} = 101.24$  ( $df = 15$ ,  $p < 0.001$ );  $\chi^2_{(3)} = 284.86$  ( $df = 15$ ,  $p < 0.001$ );  $\chi^2_{(4)} = 141.60$  ( $df = 13$ ,  $p < 0.001$ ). The cumulative heterogeneity at the four loci is also very high (Table

TABLE 2.

Heterogeneity of frequencies of prevailing alleles among different population units of the pink shrimp *Pandalus borealis*.

Population Unit	$\chi^2$	df	Significance (p<)	N
Locus Pgm				
JS	4.01	5	0.9	452
BS	2.56	5	0.9	467
BR	1.90	2	0.5	288
JS-BS	138.45	11	0.001	919
JS-BR	204.46	8	0.001	740
BS-BR	48.71	8	0.001	755
Totally for all samples	307.39	15	0.001	1272
Locus Gpi				
JS	1.82	5	0.9	457
BS	7.48	5	0.5	469
BR	1.94	2	0.5	288
JS-BS	62.98	11	0.001	926
JS-BR	40.54	8	0.001	745
BS-BR	9.81	8	0.5	757
Totally for all samples	101.24	15	0.001	1256
Locus Mdh				
JS	5.89	5	0.5	440
BS	9.58	5	0.1	469
BR	0.05	2	0.99	284
JS-BS	207.78	11	0.001	909
JS-BR	225.75	8	0.001	724
BS-BR	15.18	8	0.1	753
Totally for all samples	284.06	15	0.001	1520
Locus Fdh				
JS	6.35	4	0.5	307
BS	9.97	6	0.1	457
BR	4.90	2	0.1	253
JS-BS	98.85	10	0.001	764
JS-BR	35.64	7	0.001	440
BS-BR	15.19	8	0.1	710
Totally for all samples	141.60	13	0.001	1017
All loci				
JS	18.07	19	0.9	-
BS	29.59	20	0.1	-
BR	8.79	8	0.5	-
JS-BS	508.06	43	0.001	-
JS-BR	506.39	31	0.001	-
BS-BR	88.89	32	0.001	-
Totally for all samples	834.29	58	0.001	-

Note: Chi-square comprises the results of Workman-Niswander's test for heterogeneity (Workman, Niswander 1970); df is the number of degrees of freedom; N—number of animals in the sample.

2). When comparing different population units pooled in pairs, the shrimp from the Barents Sea clearly showed less heterogeneity than those from the Bering Sea (Table 2).

The criteria used above for heterogeneity do not allow us to compare the relationship of the variability within one sea basin to those between different seas, or to the relative differences among

samples in the whole sample set. To do this we used the minimal genetic distance,  $D_m$ . As expected, the minimal distance occurred between sample pairs from the same sea, and the maximal distances occurred between pairs of samples from different seas. The average  $D_m$  values calculated for all population units and their combinations consistently demonstrated this pattern (Table 3). In the first two columns of the table are the population genetic differentiation measured by  $D_{ST}$  and  $F'_{ST}$  (Nei 1987). The results obtained by all three statistics,  $D_{ST}$ ,  $F'_{ST}$  and  $D_m$ , support the same conclusions: shrimps from one sea are the least differentiated, those from different seas are the most differentiated. The pooled average values  $D_{ST}$ ,  $F'_{ST}$  and  $D_m$  totaled for four polymorphic loci are: (a) within a sea—0.000931, 0.004485, and 0.000319 respectively; and (b) between different seas—0.009208, 0.033164 and 0.016875 respectively (see Table 3: "within the seas" and "between the seas"). The same information is presented graphically in Fig. 2. The Barents and Bering seas populations are apparently less differentiated than the other pairs of population units (Table 3, Fig. 2).

By use of the discriminant analysis technique, it is possible to estimate the accuracy of the classification of the shrimp samples to one or another sea basin. The discriminative accuracy of the method came to 100 percent when classification was made on the basis of sample allele frequencies at all four polymorphic loci, i.e. the samples were exactly classified to their "native" populations or stocks of separate sea basins (Fig. 3). Two samples taken in 1987, which were not examined at the Fdh locus, were omitted in this analysis. Fig. 3 shows that the three clusters JS, BS and BR do not overlap. Population units, such as BR and BS pink shrimp, are closer to each other than to the JS population unit when the values of first canonical variables ( $CV_1$ ) are considered. This is in accord with our previous results.

Of independent interest for us is the problem of subdivision among different population units. The subdivision implies certain isolation between local shrimp collections or some restrictions of the gene flow. We estimated the total size of the immigrant exchange rate ( $Nm$ ) between the local shrimp populations of different Far Eastern seas derived from  $F'_{ST}$  as  $Nm = 5$  (Kartavtsev et al. 1991a). Taking into account the overlapping of the generations of the pink shrimp, the real exchange between some populations can be four-five times less, and does not exceed one individual per generation. Our results also indicate that the exchange level should be higher between the BR and BS population units. Using the approach described in Slatkin (1985), and taking the  $F'_{ST}$  estimates averaged over all four loci (Table 3), we can derive the exchange rate  $Nm$  for this pair of populations. Taking into account the generation overlap, the estimated value is:  $Nm = 33/4 = 8$ ; i.e. the exchange rate is 8 individuals per generation.

Populations of pink shrimp may reach large numbers. But in Arctic waters the effective-and-reproductive population size,  $N_e$ , may be rather small if there are no other neighboring populations and if one takes into consideration the complete absence of females in many gatherings (Berenboim 1982). From new estimates on differentiation induced by gene drift (Allendorf and Phelps 1981), one can consider that a divergence of the Barents Sea shrimp from those inhabiting the Bering Sea is due to a random process, despite some migration flow between them.

There is another explanation. If the conditions of high latitudes everywhere exert similar pressures of natural selection on allozyme polymorphism, then the  $F'_{ST}$  values calculated using the allele frequencies are underestimated, while the exchange rate is

TABLE 3.  
Estimations of population genetic differentiation in the shrimp *Pandalus borealis*.

Population Unit	$D_{ST}$	$F'_{ST}$	$\bar{D}_m \pm \bar{SE}$	n	k
Locus Pgm					
JS	.001603	.003772	-.000567 ± .007680	15	6
BS	.000427	.002764	-.000191 ± .000280	15	6
BR	.000025	.002515	-.000001 ± .000000	3	3
Totally	.016382	.069758	.030946 ± .016781	120	16
Locus Gpi					
JS	.000166	.001288	-.000821 ± .000110	15	6
BS	.000017	.005025	.000016 ± .000000	15	6
BR	.000008	.002506	-.000001 ± .000000	3	3
Totally	.001126	.021391	.001792 ± .001760	120	16
Locus Mdh					
JS	.000982	.004252	-.000091 ± .003378	15	6
BS	.003151	.006540	.002991 ± .009549	15	6
BR	.000036	.000072	-.002582 ± .002777	3	3
Totally	.028277	.067613	.053467 ± .026570	120	3
Locus Fdh					
JS	.001908	.008469	.002416 ± .003677	10	5
BS	.000219	.006632	.000248 ± .000000	15	6
BR	.000173	.005173	.000124 ± .000000	3	3
Totally	.002926	.028145	.004935 ± .003815	91	14
Average at Pgm, Gpi, Mdh					
JS	.000917	.003104	-.000493 ± .003723	45	—
BS	.001198	.004776	.000939 ± .003276	45	—
BR	.000023	.001698	-.000861 ± .000926	9	—
JS-BS	.012644	.041712	.023395 ± .013317	198	—
BS-BR	.001612	.008164	.001951 ± .003860	108	—
JS-BR	.017975	.057497	.034096 ± .014859	108	—
Within the seas	.000963	.003736	.000124 ± .003266	99	—
Between the seas	.011156	.037078	.020592 ± .011252	414	—
Totally	.015262	.052921	.028735 ± .015037	360	—
Average at Pgm, Gpi, Mdh, Fdh					
JS	.001097	.004079	.000036 ± .003714	55	—
BS	.000954	.005240	.000766 ± .002457	60	—
BR	.000060	.002566	-.000615 ± .000694	12	—
JS-BS	.010619	.038541	.019517 ± .011314	253	—
BS-BR	.001254	.007482	.001503 ± .002895	144	—
JS-BR	.015005	.050354	.028238 ± .012699	136	—
Within the seas	.000931	.004485	.000319 ± .002835	127	—
Between the seas	.009208	.033164	.016875 ± .009393	533	—
Totally	.012773	.047922	.023933 ± .012773	451	—

Note: JS, BS, BR—shrimps from Sea of Japan, Bering Sea and Barents Sea;  $D_{ST}$  and  $F'_{ST}$  are the measures of genetic differentiation among populations (Nei 1987);  $\bar{D}_m$ —unbiased minimal genetic distance (Nei 1978); n—number of pair-wise comparisons; k—number of samples (sub populations). Mean errors (SE) are calculated from the variance matrices.

overestimated. The lowest heterozygosity of shrimps taken from the Barents Sea and the reduced heterozygosity of those from the Bering Sea (Table 1, see  $H_d$ ) may be explained by the limits of adaptive strategy of the high latitude "specialists" (Valentine 1976) and by the bottleneck effect when the Pacific shrimp expanded into the Arctic and Atlantic basins. In any case, the causes of the low genetic differentiation in the shrimp populations of Bering and Barents Seas can not now be identified without additional data, including both more polymorphic markers and wider sampling over the species area. The low genetic differentiation was surprising. *A priori* we reasoned that since the last glaciation, i.e. some 0.5 million years ago, the Atlantic and Barents Sea

populations and Pacific ones were isolated. This must have provided time for accumulation of several different allelic mutations.

We conclude that:

1. Groups of gatherings or local populations of the pink shrimp in the Barents Sea, Bering Sea and Sea of Japan are self-reproducing and appear to be panmictic units.

2. The pink shrimp from the Barents Sea are most isolated from the shrimp inhabiting the Sea of Japan.

3. The least population genetic differentiation has been found between shrimp from the Barents Sea and those from the Bering Sea. The reasons are for this low differentiation of the northern populations are not clear.

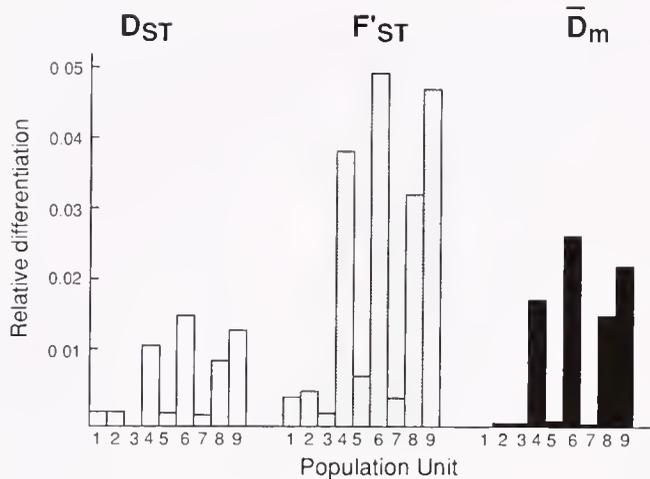


Figure 2. Values of relative gene pool differentiation in different population units of the pink shrimp *Pandalus borealis*.

The  $D_{ST}$ ,  $F'_{ST}$ ,  $\bar{D}_m$  ranges are marked on left scale. The abbreviations below designate as follows: 1—JS, 2—BS, 3—BR, 4—JS-BS, 5—BS-BR, 6—JS-BR, 7—within seas, 8—between seas, 9—total value.

Our conclusions are generally consistent with results published elsewhere. Investigators of population structure of shrimp in the Barents Sea (Berenboim 1982, Teigsmark 1983) suppose that there is only one (or a few) independent populations there and others show differing degrees of dependence. The whole set of populations in the Barents Sea may be considered as one superpopulation (Berenboim 1982). The results presented here and previously (Kartavtsev et al. 1991a) agree well with this notion. At the same time, the gatherings within a sea can be rather distinctly differentiated both morphologically and ecologically (Berenboim

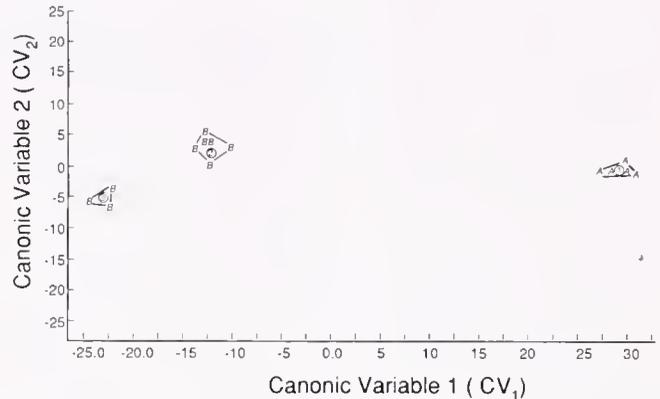


Figure 3. Distribution of samples of pink shrimp *Pandalus borealis* examined at four loci in the scale of canonic variables (CV).

First canonic variable— $CV_1$ , second canonic variable— $CV_2$ . Circles with numbers 1–3 denote average CV-values for JS, BS and BR population units respectively. Each letter indicates a sample.

1978, 1982, Teigsmark 1983, Shumway et al. 1985, Kartavtsev et al. 1991b).

The  $D_{ST}$ ,  $F'_{ST}$  and  $D_m$  values listed in Table 3 exhibit relatively little genetic difference, even between very distant geographical populations of the pink shrimp. Similar results have been reported for some other crustaceans as well, including various shrimp species (Hedgecock et al. 1982, Chow and Fujio, 1985, 1988) and Euphausiids (Fevolden and Ayala 1981, McDonald et al. 1985). They were explained by the high migration capacity of these species possessing a prolonged planktonic larval stage. We, too believe that it is the interpopulation exchange that is responsible for the low total genotype differentiation in species with such a biology.

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**A BIBLIOGRAPHY OF "FRESHWATER CRAYFISH: A JOURNAL OF ASTACOLOGY I-VII",  
THE PROCEEDINGS OF THE INTERNATIONAL ASSOCIATION OF  
ASTACOLOGY SYMPOSIA  
1973-1988**

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**ABSTRACT** A bibliography of over 300 papers published in the International Association of Astacology journal "Freshwater Crayfish: A Journal of Astacology" from 1973 to 1988 is given so that this valuable source of information on the biology of freshwater crayfish is made more widely known.

**KEY WORDS:** freshwater crayfish, bibliography, Astacidae, Cambaridae, Parastacidae.

**INTRODUCTION**

The International Association of Astacology was founded in Austria in 1972. The aims of the association are to encourage the scientific study of crayfish for the benefit of mankind, to provide for dissemination of research findings, and to develop an international forum for the free discussion of problems relevant to crayfish. Regular symposia are held and these are published as "Freshwater Crayfish—A Journal of Astacology." Volumes I-VII contain over 300 papers. Freshwater Crayfish I was published in 1973 and relates to the first symposium held in Hinterthal, Austria in 1972. It was edited by S. Abrahamsson. Information about subsequent volumes follows: II—1975, Baton Rouge, Louisiana, USA 1974, ed. J. W. Avault, Jr.; III—1977, Kuopio, Finland 1976, ed. O. V. Lindqvist; IV—1979, Thonon-les-Bains, France 1978, ed. P. J. Laurent; V—1983, Davis, California, USA, 1981,

ed. C. R. Goldman; VI—1986, Lund, Sweden 1984, ed. P. Brinck; and VII—1988, Lausanne, Switzerland 1987, ed. P. Goeldlin de Tiefenau. Volume VIII relates to the symposium held in Baton Rouge, Louisiana, USA 1990 and is currently being edited by R. P. Romaine.

As these volumes contain such a wealth of information on crayfish which has never been catalogued, it was decided to publish the information for the benefit of all crustacean biologists. Currently, Volumes I and IV through VII are available for purchase details of which can be obtained from Dr. J. V. Huner, I.A.A. Secretariat, Crawfish Research Center, University of Southwestern Louisiana, Lafayette, Louisiana, 70504, USA, as can information about joining the I.A.A.

The ninth (IX) symposium of the I.A.A. will be held at Reading University, England, from April 5-10 in 1992, further details of which can be obtained from the author.

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## EVALUATION OF TWO NEW TRAP TYPES AND AERATOR-INDUCED WATER CURRENTS FOR HARVESTING PROCAMBARID CRAWFISH IN PONDS<sup>1</sup>

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**ABSTRACT** Three 2-ha experimental crawfish ponds were used to evaluate water circulation and two new trap types to enhance crawfish (*Procambarus sp.*) harvesting efficiency. Paddlewheel aerators were placed in baffle channels to create water currents, and hoop traps, box traps and conventional traps were evaluated and compared.

Un-baited hoop traps ( $\bar{x}$  = 203 crawfish/trap and 7.1 kg/trap) were more effective at harvesting than un-baited box traps ( $\bar{x}$  = 58 crawfish/trap and 1.8 kg/trap) and baited conventional traps ( $\bar{x}$  = 37 crawfish/trap and 1.0 kg/trap). Hoop and box traps located inside baffle channels ( $\bar{x}$  = 115 crawfish/trap and 3.2 kg/trap) caught 5.5 and 6.4 times, respectively, more crawfish than similar traps located in open areas. Hoop traps set for 48-h caught significantly more crawfish than those set for 24-h. Crawfish catch was not different in traps facing upstream vs facing downstream.

**KEY WORDS:** crawfish, *Procambarus*, aquaculture, aquaculture engineering, harvest

### INTRODUCTION

The methods used to harvest crawfish differ significantly from those used for other cultivated aquatic species. Most cultivated aquatic animals are harvested from one to several times per production cycle with nets or seines, by draining the pond, or with a combination of both. In contrast, in the southern United States crawfish of the genus *Procambarus* are cultivated in shallow, open ponds in which vegetation is grown as a detrital forage for the crawfish. The vegetation hinders harvest with seines or similar devices, and batch harvest is not practiced. Rather, crawfish are caught with small, baited wire traps which are emptied from 60 to 150 days during the production cycle. They are typically harvested from ponds beginning in late November, approximately 6-8 weeks after the ponds are flooded in September or October. Traps are baited and emptied 4-6 days weekly, continuing through May or June when the ponds are drained (Romaine 1989). An experienced crew of two people can empty 150-300 traps per h depending on the type of trap and boat used.

Current crawfish harvesting practices are inefficient, labor intensive, and costly. Annual harvest expenses incurred by farmers normally range from \$1,250-1,500 per ha, and as much as 60-80 percent of total production costs are associated with harvest (Dellenbarger et al. 1987, Pomeroy and Kahl 1987). Improvements in harvesting technology could increase crawfish production efficiency and decrease costs. Ideally, crawfish should be harvested with minimal use of traps, bait and labor. Methods of harvesting cambarid crawfish and factors that influence harvest are reviewed by Romaine (1989).

A harvest method using water currents generated by paddlewheel aerators to attract crawfish into swiftly moving water currents and trap them with hoop or box traps has been evaluated experimentally and is the subject of this paper. The objectives of

this study were (1) to evaluate a recirculating pond configuration wherein crawfish could be stimulated by water currents generated by paddlewheel aerators to migrate into confined areas for ease of harvest; and (2) to evaluate newly developed hoop and box traps for harvesting crawfish and to compare them with a conventional commercial crawfish trap.

### METHODS AND MATERIALS

#### *Pond Design and Management*

Three crawfish ponds, each 1.8 to 2.0-ha in surface area, and located at the Ben Hur Research Farm, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, were used in this project. The ponds contained resident populations of red swamp crawfish (*Procambarus clarkii*), but broodstock was supplemented with 75 kg/ha of mature crawfish in June 1988. Rice (*Oryza sativa*, Mars variety) was planted in the ponds on 16 August 1988 as forage for the crawfish, and the ponds were flooded to a mean water depth of about 45 cm from 1-15 October to stimulate young-of-the-year to emerge from burrows. Each pond was plumbed with a water inlet and outlet for filling and draining, and cascade aerators using three screens fabricated from 12.7-mm mesh expanded metal were used to aerate well water as the ponds were filled (Lawson et al. 1984). Crawfish cultivation practices followed those recommended to commercial producers in Louisiana (de la Bretonne and Romaine 1989).

The research pond design is illustrated in Figure 1. Each pond was constructed with an earthen baffle levee network for internal recirculation of the water with aid of paddlewheel aerators. Aerators were placed between parallel pairs of levees spaced 7.8-m apart (S) which formed "channels" in interior sections of each pond. The channels created were trapezoidal in shape with a mean cross-sectional area of 3.1-m<sup>2</sup>.

#### *Paddlewheel Aerators*

Paddlewheel aerators (PA) were used to generate water currents because they have excellent ability to circulate water horizontally in shallow ponds (Rogers 1990), and they are efficient in oxygen transfer (Jensen and Bankston 1988). Two 2.2-kW, 115

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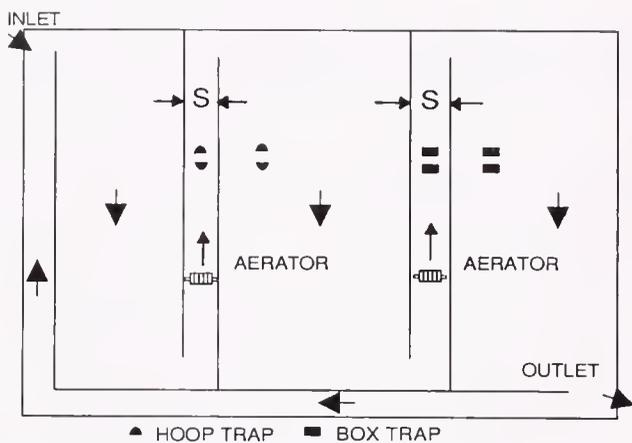


Figure 1. Pond configuration used in 2-ha experimental crawfish ponds. Arrows indicate direction of water flow created by the paddle-wheel aerators.

VAC single-phase Airoflo paddlewheel aerators (S & N Sprayer Co., Inc., Greenwood, MS) were installed in each pond (Fig. 1). For optimum water flow and oxygen transfer the aerators were operated at the manufacturer-recommended paddle depth of 19-cm and rotational speed of 83 rpm, and the units were operated continuously during the trials. A detailed description of the aerator design is provided by Lawson et al. (1991). Water temperature and dissolved oxygen concentration were determined between 0700-0900 h with a polarographic oxygen meter with thermistor (YSI Model 57 or 58, Yellow Springs, OH) during the harvest trials.

#### Trap Designs

The hoop trap (HT) design was modified from a commercial hoop net used to harvest cambarid crawfish in Spain (Gaudet 1983). The experimental HT were rigid, cylindrical in shape and built from 19-mm hexagonal mesh PVC-coated wire (Fig. 2). They were 1.8-m long, 0.5-m in diameter at the entrance, and had three 23-cm long successively smaller internal funnels which extended inward from the trap walls at a 45° angle. The funnel opening diameters were 13, 9 and 6-cm, respectively. The traps were flattened slightly on one side so that they would lay flat when placed horizontally on the pond bottom. The HT were 75–100 percent submerged when in use. The "cod end" of the trap could

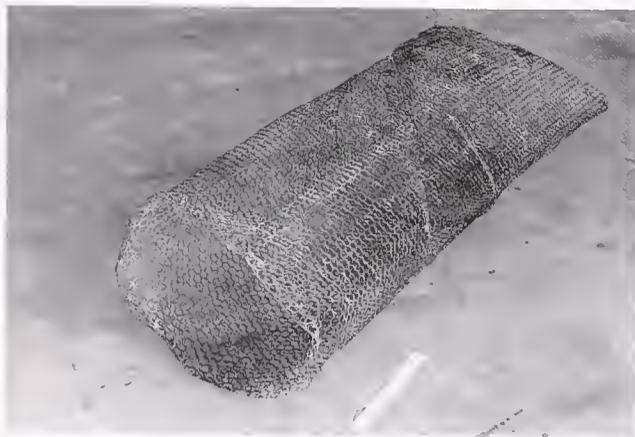


Figure 2. Experimental hoop trap used in the study. Size reference is indicated by the 30.5-cm (12-in) rule placed alongside the trap.

be opened to remove the crawfish. Each HT weighed less than 2 kg and required only one person to lift and empty.

Experimental box traps (BT) were fabricated from 9.5-mm diameter steel rod and measured 1.0-m long by 0.6-m deep. Trap height tapered from 0.5-m at the upstream face to 0.3 m on the downstream face (Fig. 3). PVC-coated wire (19-mm hexagonal mesh) was attached to the outside of the frame with plastic tie-wraps. A barrier on the upstream side extended from the top of the trap to above the water surface to prevent crawfish from swimming over the trap. Crawfish entered through a 0.6-m wide by 7.5-cm high opening on the upstream face of the trap. The opening tapered to 5-cm high on the inside. The bottom edge of the trap entrance was located 7.5-cm up from the bottom of the trap. A "ramp" of PVC-coated hex wire, placed on the pond bottom in front of the trap, led crawfish upwards into the opening. Each box trap weighed about 35-kg and required 2 persons to lift and empty.

The performance of the HT and BT was compared to a commercial stand-up pillow trap (Fig. 4). The conventional traps (CT) were oval-shaped, 36-cm diameter, and approximately 61-cm high. They had two entrance funnels at the base for crawfish to enter, and were open at the top to facilitate emptying and re-baiting. A 7.6-cm wide smooth aluminum band was attached around the inside of the top opening to prevent crawfish from escaping. To prevent the traps from tipping over by wind forces a 9.5-mm diameter steel rod attached to the side of the trap was pushed into the soft pond bottom when the traps were set.

#### Trap and Aerator Placement

Four HT, 4 BT and 16 CT were placed in each of the three ponds. In each pond 2 HT were placed back-to-back in one baffle channel with one facing upstream and the other positioned downstream to the water current. Two BT were placed similarly in the second baffle channel. The remaining 2 HT and 2 BT and the 16 CT were placed in open regions of the ponds (Fig. 1). No attractant (bait) was used in the HT and BT during the study but 150 g of manufactured crawfish bait was added to each CT during trap evaluations. Fresh bait was used on each sampling date. The crawfish trapping studies were conducted in May 1989.

Two paddlewheel aerators were operated in series in each pond (Fig. 5). Water velocity in the channels and open areas was determined with a velocity meter (Model 202, Marsh-McBirney, Inc., Frederick, MD). Cross-sectional areas of the channels and open pond sections were measured, and water flow rate,  $Q$ , was

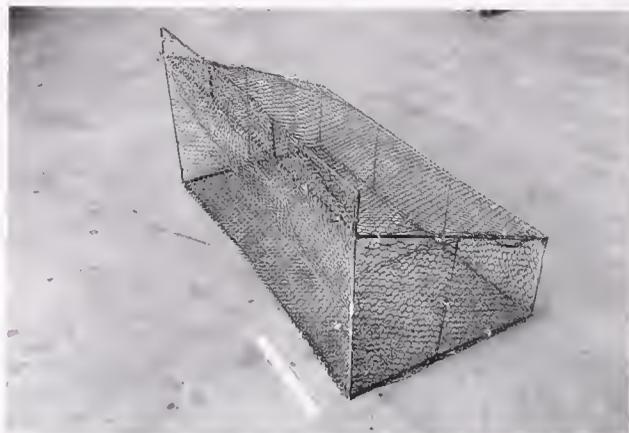


Figure 3. Experimental box trap used in the study.

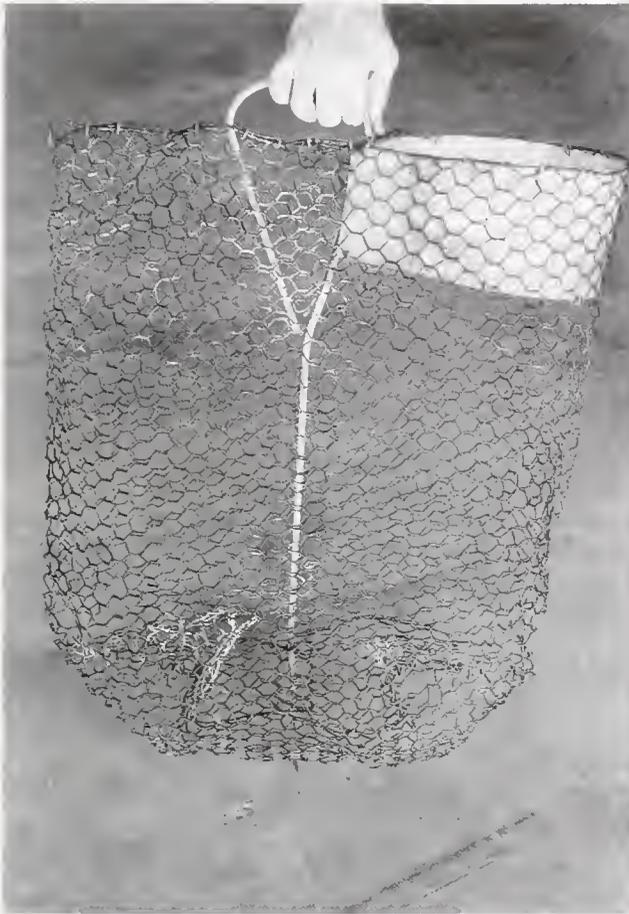


Figure 4. A commercial stand-up pillow trap used for harvesting crawfish in ponds.

calculated from (Simon 1981):  $Q = A * V$  where  $Q$  = water flow rate,  $m^3/min$ ;  $A$  = cross-sectional area,  $m^2$ ; and  $V$  = velocity,  $m/min$ .

#### Comparison of Three Trap Designs and Trap Set Time

Crawfish catch and size was evaluated for HT, BT, and CT in both 24-h and 48-h trap-sets on 8 sampling dates in each of the



Figure 5. One of the 2.2-kW (3-hp) paddlewheel aerators used in the study.

three ponds. Crawfish in each trap were counted, and the total weight obtained. Trap-set time (i.e., trap-soak time) is the time elapsed between setting the trap and removing the catch. Mean size was determined by dividing total weight of crawfish per trap by number caught per trap. After initial trials demonstrated that BT were not as effective as HT in capturing and retaining crawfish another trial was conducted to evaluate 24-h and 48-h set times only with HT located in the channels where water velocity was highest.

#### Trap Location and Trap Orientation

The catchability of crawfish in HT and BT in water of "high" velocity versus "low" velocity, and the effect of HT and BT orientation on crawfish harvest was evaluated on 7 sampling dates. The crawfish captured in HT and BT located in the channels where water velocity was high was compared to harvest in open areas of the ponds where velocity was low. The HT and BT in both channel and open areas were oriented so that the entrance of one trap of each type faced upstream and the other downstream. Conventional traps were not included in this experiment because they were not designed to be placed in channels nor could the entrances on the CT be positioned relative to current direction.

#### Crawfish Escapement

Twenty marked crawfish were placed inside eight HT and BT, and the number of crawfish that escaped after 24 and 72-h was determined.

#### Data Analysis

Statistical analysis of trapping data was conducted with the Statistical Analysis Systems software using the General Linear Models Test (GLM) Procedure (SAS Institute 1982). The experimental design was a completely randomized block design with pond as the block in the main effect of the model. Sampling date was incorporated as a split-plot component (repeated measures design) in the statistical model. The main treatment effects in the statistical models included either trap design (HT, CT, BT), trap location (open vs channels); trap orientation (upstream vs downstream), and trap-soak time (24-h vs 48-h), and the respective first order and second order interactions of the main effects. Response variables included number of crawfish caught per trap, weight of crawfish per trap, and mean size of crawfish captured. Duncan's New Multiple Range Test was used to determine if statistical differences existed between treatment means for trap designs, and differences in treatment means were declared to be significant at  $\alpha = 0.05$ .

## RESULTS

#### Aerator Performance

The mean water velocity generated by two aerators in series during the harvest study was 0.08 m/s in the channels compared to 0.02 m/sec in open areas. Mean water circulation rate was 10.8  $m^3/min$ , resulting in an average water recirculation rate (turnover) of once every 15-h. The current generated by the aerators was visually apparent in the channels but not so in the open areas. Water temperature ranged between 26 and 32°C, and dissolved oxygen concentrations remained above 3 mg/liter during the course of the study.

### Trap Type and Trap-Set Time

The mean crawfish catch (number and weight/trap) by trap types and set times is displayed in Table 1. Catch was highest in pond D2 ( $\bar{x}$  = 145 crawfish and 6.1 kg/trap) followed by D1 ( $\bar{x}$  = 96 crawfish and 1.7 kg/trap). Mean catch was lowest in D4 ( $\bar{x}$  = 67 crawfish and 2.3 kg/trap). The mean size of crawfish captured averaged over the three trap types was 44.2-g in D2, 35.1-g in D4 and 17.1-g in D1. Over 98% of the crawfish harvested were red swamp crawfish, *Procambarus clarkii*, with the remainder being white river crawfish, *P. zonangulus*. Data for the two species were combined for analysis.

The number and total weight of crawfish caught per trap-set were significantly higher in HT ( $P < 0.05$ ). There were no differences in catch between BT and CT ( $P > 0.05$ ; Table 1). The HT ( $\bar{x}$  = 212 crawfish/trap and 7.1 kg/trap) captured 3.7 and 3.9 times more crawfish, by number and weight, respectively, than BT ( $\bar{x}$  = 58 crawfish/trap and 1.8 kg/trap) and 5.7 and 7.1 times more than CT ( $\bar{x}$  = 37 crawfish/trap and 1.0 kg/trap) ( $P < 0.05$ ). Crawfish captured in HT and CT ( $\bar{x}$  = 30.5 g) were smaller in mean size than those captured in BT ( $\bar{x}$  = 35.6 g) ( $P < 0.05$ ). The highest crawfish catch observed in any one trap on any sampling date was as follows: HT = 28.0 kg/trap, BT = 11.5 kg/trap and CT = 2.7 kg/trap.

A significant statistical interaction was present between pond, trap type and trap-set time. In the ponds with the lowest crawfish density (D1) there was no difference in crawfish catch between the 24-h and 48-h trap sets ( $P > 0.05$ , Table 1). However, in the ponds with higher populations of harvestable sized crawfish (D2, D4), the catch of crawfish in HT increased significantly from 24-h to 48-h trap sets (from 298 to 381 in D2 and from 139 to 186 in D4). By comparison, the catch in BT decreased significantly with 48-h trap sets compared to a 24-h trap sets in both D2 and D4. There was no difference between 24-h and 48-h sets for BT in pond D1 ( $P > 0.05$ ). In addition, there was no difference in number caught or weight/trap for CT among ponds ( $P > 0.05$ ).

### Trap Location and Orientation

Catch data for HT and BT located in channels vs open areas and traps facing upstream vs downstream are shown in Table 2. Conventional traps were not used in channels and were, therefore, not included in these comparisons. The combined mean catch for HT and BT located inside baffle channels where water velocity was higher ( $\bar{x}$  = 115 crawfish/trap and 3.2 kg/trap) was significantly higher (5.5 and 6.4 times, respectively) than those placed in open pond areas ( $\bar{x}$  = 21 crawfish/trap and 0.5 kg/trap;  $P < 0.05$ ),

TABLE 2.

Mean ( $\pm$ SD) number and weight of crawfish harvested with hoop traps (HT) and box traps (BT) in three experimental crawfish ponds on 7 sampling dates.

Location	Number Crawfish/Trap		Weight/Trap (kg)	
	US <sup>a</sup>	DS <sup>b</sup>	US	DS
-----Hoop Traps-----				
CH <sup>c</sup>	191 $\pm$ 176	146 $\pm$ 202	4.6 $\pm$ 5.1	5.0 $\pm$ 7.1
OP <sup>d</sup>	20 $\pm$ 15	32 $\pm$ 77	0.5 $\pm$ 0.4	0.6 $\pm$ 1.1
-----Box Traps-----				
CH	61 $\pm$ 90	61 $\pm$ 55	1.1 $\pm$ 1.3	2.0 $\pm$ 2.8
OP	17 $\pm$ 18	15 $\pm$ 10	0.4 $\pm$ 0.3	0.3 $\pm$ 0.2

<sup>a</sup> US = Traps facing upstream.

<sup>b</sup> DS = Traps facing downstream.

<sup>c</sup> CH = Traps located in channels.

<sup>d</sup> OP = Traps located in open areas.

indicating that crawfish were migrating into baffle channels as theorized. HT in channels ( $\bar{x}$  = 168 crawfish/trap and 4.8 kg/trap) caught significantly more crawfish (2.7 and 3 times, respectively) than BT in channels ( $\bar{x}$  = 61 crawfish/trap and 1.6 kg/trap;  $P < 0.05$ ). Mean size of crawfish caught in both HT and BT did not differ between channels ( $\bar{x}$  = 28.8 g) and open areas ( $\bar{x}$  = 27.0 g;  $P > 0.05$ ).

Trap orientation had no effect on number, weight or size of capture ( $P > 0.05$ ). The combined mean catch in the channels with HT and BT facing upstream was 126 crawfish/trap and 2.8 kg/trap compared to 103 crawfish/trap and 3.5 kg/trap in those facing downstream. The crawfish appeared to have no significant preference for migrating with or against the current at water velocities generated in this study.

### Separate Hoop Trap Studies

Because HT was the most efficient of the three trap types evaluated, we conducted additional studies using HT only. The effectiveness of HT in channels vs open pond areas and HT oriented upstream vs downstream was compared on 5 sampling dates (Table 3). HT located in channels caught more crawfish ( $\bar{x}$  = 176 crawfish/trap and 4.8 kg/trap) than those in open areas ( $\bar{x}$  = 19 crawfish/trap and 0.5 kg/trap;  $P < 0.05$ ). Catch of crawfish with HT facing upstream did not differ from downstream ( $P > 0.05$ ). Additionally, there was no significant difference in crawfish size in HT with respect to location or orientation ( $P > 0.05$ ; Table 3).

TABLE 1.

Mean number, weight, and size of crawfish harvested per trap for 24 and 48-h trap set times for hoop traps (HT), box traps (BT) and conventional traps (CT) in three experimental crawfish ponds on 7 sampling dates.

Pond	Set Time (hours)	Number Harvested			Weight Per Trap (kg)			Size (g)		
		HT	BT	CT	HT	BT	CT	HT	BT	CT
D1	24	137	98	56	2.6	1.5	0.9	19.0	15.3	16.1
	48	131	94	58	2.4	1.6	1.0	18.3	17.0	17.2
D2	24	298	80	25	12.0	4.3	1.0	40.3	53.8	40.0
	48	381	53	32	14.9	2.9	1.2	39.1	54.7	37.5
D4	24	139	15	31	4.8	0.5	1.1	34.5	33.3	35.5
	48	186	7	21	6.2	0.3	0.7	33.3	39.4	34.5

TABLE 3.

Mean number, weight, and size of crawfish caught with hoop traps (HT) in three experimental crawfish ponds on 5 sampling dates.

Location	Orientation	Number Crawfish per Trap	Weight/Trap (kg)	Size (g)
CH <sup>a</sup>	US <sup>b</sup>	172	4.6	26.7
CH	DS <sup>c</sup>	179	5.0	27.9
OP <sup>d</sup>	US	16	0.5	30.6
OP	DS	21	0.6	28.0

<sup>a</sup> CH = Traps located in channels.

<sup>b</sup> US = Traps facing upstream.

<sup>c</sup> DS = Traps facing downstream.

<sup>d</sup> OP = Traps located in open area.

Table 4 shows the results of a study conducted on 7 sampling days comparing upstream vs downstream orientation and 24-h vs 48-h trap-set times for HT located in channels. Mean catch in HT located in the channels after a 48-h set ( $\bar{x}$  = 127 crawfish/trap and 3.7 kg/trap) was 1.5 times higher in number and 1.7 times higher in weight/trap than in 24-h sets ( $\bar{x}$  = 81 crawfish/trap and 2.2 kg/trap) ( $P < 0.1$ ). There was no difference in catch between HT facing upstream ( $\bar{x}$  = 104 crawfish/trap and 3.0 kg/trap) and those facing downstream ( $\bar{x}$  = 104 crawfish/trap and 3.0 kg/trap) ( $P > 0.05$ ).

#### Crawfish Escapement

Four percent of the marked crawfish placed in HT escaped after 24-h, and 99 percent escaped after 72-h. In contrast, 70 percent of crawfish entering the BT escaped after 24-h and 99 percent escaped after 72-h.

#### DISCUSSION

This study has demonstrated that a pond configuration using internal baffle levees constructed within the confinement of perimeter levees is effective for circulating water in crawfish ponds. Paddlewheel aerators are acceptable for use in crawfish ponds because they provide good aeration and water circulation when operated at the recommended rotational speed and paddle depth. The paddlewheel aerators were effective in maintaining a recommended oxygen concentration of 3 mg/liter or greater (Romaine 1985) in the experimental ponds throughout the study period. Both horizontal and vertical water movement is important in crawfish aquaculture because crawfish do not swim long distances to oxygenated zones as can finfish during periods of oxygen depletion

TABLE 4.

Mean number, weight, and size of crawfish caught with hoop traps (HT) in channels for 24-h and 48-h set times in three experimental crawfish ponds on 7 sampling dates.

Orientation	Set Time (hours)	Number Crawfish per Trap	Weight/Trap (kg)	Size (g)
US <sup>a</sup>	24	91	2.6	28.5
US	48	116	3.4	29.4
DS <sup>b</sup>	24	70	1.9	27.3
DS	48	138	4.1	29.7

<sup>a</sup> US = Traps facing upstream.

<sup>b</sup> DS = Traps facing downstream.

(Lawson et al. 1991). Thus, oxygenated water must be circulated throughout the pond as opposed to creating localized oxygenated zones around the aerator to which the animals must migrate for survival. Many aerator designs used in finfish ponds do not effectively move water horizontally. No mechanical failures of the units occurred in 2 months of continuous operation. Maintenance consisted of weekly greasing paddlewheel aerator bearings.

The un-baited HT in channels were several times more efficient than either un-baited BT in channels or baited CT. The BT were not effective because a high percentage of the crawfish that entered the traps escaped within 24-h of setting the trap. This likely explains why BT caught larger crawfish than HT and CT. Larger crawfish have greater difficulty escaping through entrances than do smaller crawfish. High numbers of crawfish escaped from HT after 48-h. Modifications in the HT and BT designs should be possible to reduce the rate of crawfish escape through the entrances while allowing crawfish to continue to enter the trap with minimum impediment. Because of their smaller size and weight the HT were physically easier to set and empty than the BT.

We thought prior to initiation of this study that crawfish would be attracted into channels where water velocity was higher and better aerated, and this was demonstrated. The aerator-generated currents stimulated significant numbers of crawfish to move to baffle channels presumably because water quality was better in the channels and/or because of an affinity of *Procambarus clarkii* to position themselves in areas of higher water velocity. Capture efficiency of HT and BT were significantly increased when they were set in baffle channels compared to poor performance in open areas of the ponds. In fact, the catch with un-baited HT and BT placed in open pond areas was either equal to or less than the smaller CT placed nearby.

The HT and BT were not baited in the study because bait typically comprises 40 to 60 percent of annual harvest expense (Romaine 1989), and we attempted to reduce harvest cost by reducing bait use. The use of bait in HT and BT may have increased their efficiency, but an earlier study by Baum (1987) using the same HT design as used in this study indicated that HT without bait caught the same as those with bait. Thus, we believed the addition of bait would have been an unnecessary expense.

We theorized that crawfish would tend to move against the direction of the water current, and we expected the catch to be greater in HT and BT with the entrances facing upstream. However, the data indicated that crawfish did not have a strong preference to migrate in any specific direction in either 24-h or 48-h trap-set periods at water velocities of up to 0.08 m/sec. Crawfish were as likely to be captured in traps located in baffle channels with entrances facing downstream as traps with entrances facing upstream. No bait was used in either the HT or BT, therefore, no attractant gradient was established against which the crawfish might migrate.

The catch per unit trap effort (CPUE) of the three trap types was evaluated in late spring, and these results would likely not be reflective of the CPUE in the late fall and winter. However, the standing crop of harvestable crawfish and CPUE in procambarid crawfish culture in the southern United States are greatest in late spring (Romaine 1989), and differences in catch efficiency among the various gear types would be most likely demonstrated in late spring - the period during which these trials were conducted.

Although crawfish catch by weight with un-baited HT placed in baffle channels was on average several times higher than baited CT, we believe the difference was not sufficient to justify the use of HT at present. A HT had about 16 times greater volume ca-

capacity than a CT but even at its best the HT never captured more than 10 times more crawfish (by weight) than the CT. We estimated that two un-baited HT emptied twice per week over a 24-week trapping season would have to be capable of replacing 20 CT emptied 4 times weekly to be considered as an effective alternative to present commercial trapping methods using baited CT. The HT was five times more expensive to build (labor and material), and it was logistically more difficult to lift and empty than the CT. The HT concept must be improved if it is to be feasible on a commercial scale. Additionally, ponds would have to be renovated at significant cost to the producer in order to use HT.

The results of this study lead us to conclude that crawfish ponds that are designed to recirculate water using paddlewheel aerators are effective at enhancing crawfish catch regardless of the trap design. The mean crawfish catch with CT (0.9 kg/trap) in the three ponds was about 2–2.5 times higher than what is normally ob-

served in commercial ponds in late spring (de la Bretonne and Romaire 1989). We believe that paddlewheel aerator-generated water currents stimulated crawfish activity, dispersed attractants in bait, and maintained water quality such that aerators had a positive effect in increasing the effectiveness of CT. More activity by crawfish increases the likelihood that a crawfish will come into contact with a trap.

#### ACKNOWLEDGMENTS

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## INFESTATION OF UNIONIDS BY *DREISSENA POLYMORPHA* IN A POWER PLANT CANAL IN LAKE ERIE<sup>1</sup>

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**ABSTRACT** The infestation of unionid mollusks (Unionidae) by the recently introduced zebra mussel, *Dreissena polymorpha*, and the age structure of the zebra mussel population were examined in February and August 1989 in a power plant intake canal located in western Lake Erie. In February, unionids (*Leptodea fragilis* and *Anodonta grandis*) were infested with one- (2 to 6 mm long) and two- (9 to 21 mm long) year-old mussels. Infestation intensity (i.e., number of mussels/unionid) ranged between 7 and 48/unionid and the mean intensity was 24. Between February and August, young zebra mussels were spawned and settled on unionids and on zebra mussels infesting unionids. As a result, unionids (*L. fragilis*, *A. grandis*, and *Proptera alata*) were infested by young-of-the-year (YOY; 1 to 12 mm long), one- (13 to 23 mm long), and two- (24 to 38 mm long) year-old mussels in August. Numbers of mussels ranged between 2,491 and 10,732/unionid and the mean intensity was 6,777. In August, mean dry weights of infestation were 44.8 g/unionid, representing about 74% of the weight of an average unionid. Mean weights of mussels/unionid by zebra mussel age group were 15.4 g of YOY (34% of the total), 13.4 g of one-year-olds (30%), and 16.0 g of two-year-olds (36%). Infesting zebra mussels may negatively affect unionids by disrupting unionid locomotion, metabolic activities, food availability, growth, access to substrate interface, and creating anaerobic habitat conditions. As a result, infesting zebra mussels may cause mortality of unionids in western Lake Erie and throughout North America.

**KEY WORDS:** unionids, *Dreissena*, introductions, zebra mussel

### INTRODUCTION

The zebra mussel, *Dreissena polymorpha* (Pallas 1771; Bivalvia: Dreissenidae), is believed to have been introduced into Lake St. Clair of the Great Lakes in 1986 and was firmly established (i.e., permanent population unlikely to be eliminated; Shaffland and Lewis 1984) in Lake St. Clair in 1988 (Hebert et al. 1989). By the fall of 1989, zebra mussels had spread throughout Lake Erie and were covering all available firm substrate in the western basin of the lake (Griffiths et al. 1989). In some areas of western Lake Erie, high densities in excess of 700,000/m<sup>2</sup> have been found (Griffiths et al. 1989). To date, the effects of zebra mussels on raw water users in North America have been of major concern because mussels have been identified as major biofoulers (Clarke 1952, Griffiths et al. 1989, LePage 1990). However, the environmental effects of mussels on the ecology of waters in North America have received little study because the effects have generally been considered to be of secondary importance, and because of the short period of time invading mussels have been in natural systems (Griffiths et al. 1989, Hebert et al. 1989, Leach 1990).

Studies of the zebra mussel in Europe reveal that they have the potential to substantially effect the ecology of aquatic systems, indigenous populations of aquatic organisms, and some wildlife (Lewandowski 1976, Stanczykowska 1975, deNie 1982, Draulans 1982, Borowiec 1975, Dvorak and Best 1982). In North America, Hebert et al. (1989) noted concern about the interactions between unionid mollusks (Bivalvia: Unionidae) and zebra mussels. Hebert et al. (1989) suggested that zebra mussels may be an interference competitor with unionids and cited instances of displacement of gastropods by exotic gastropods (e.g., *Bithynia tentaculata* and

*Cipangopaludina chinensis*) in the Great Lakes. The present study documents the occurrence of a zebra mussel infestation on unionids in nearshore waters of western Lake Erie. These data will aid in the evaluation of future effects of the zebra mussel on unionids in the Great Lakes and elsewhere in North America as the geographic range of the zebra mussel increases (Strayer 1991).

### METHODS

Unionids and infesting zebra mussels were collected in the forebay of a power intake canal of the Monroe Power Plant on February 15 and August 10, 1989 (Figure 1). The intake canal is located on the Raisin River about 800 m upstream from Lake Erie. The intake canal is 213 m long and 11 to 33 m wide. The canal branches to supply water to two screen houses. The sampling area was in that portion of the canal furthest from the Raisin River. At capacity, water flow entering the canal is about 5,300 m<sup>3</sup> (1.4 million gallons) per minute. Sediments in the sampled area were primarily silt and clay.

Unionids infested with zebra mussels were collected by SCUBA divers who located unionids by touch because water turbidity prevented direct observations of unionid position in the substrate. Samples were individually preserved in 5% buffered (CaCO<sub>3</sub>) formalin.

In the laboratory, zebra mussels were removed from unionids and washed over a U.S. Standard Number 60 sieve (0.25-mm mesh openings). Unionids and mussels were counted and measured to the nearest whole millimeter. Zebra mussels smaller than 1 mm long were grouped with 1 mm individuals.

Length-frequency distribution curves of zebra mussels collected in February were constructed from a random group of about one-half (i.e., 115 of 220 total) the mussels removed from unionids. Length-frequency curves of mussels collected in August were

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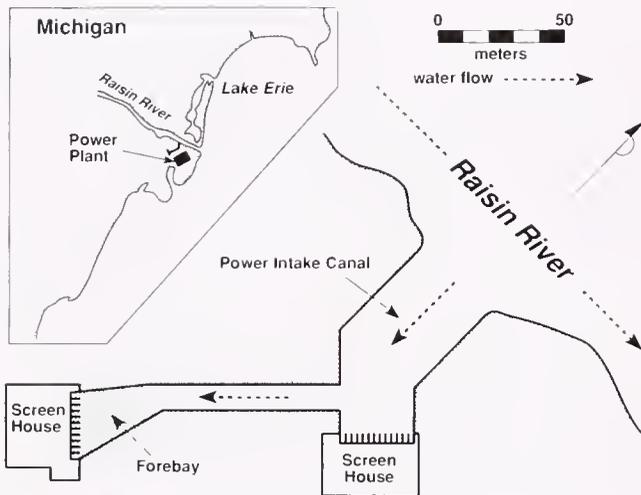


Figure 1. Monroe electric power plant along the shores of western Lake Erie. Shaded area indicates intake canal where unionid mollusks and infesting zebra mussels were collected February 15 and August 10, 1989.

based on a randomly selected sub-sample of 300 mussels  $\leq 10$  mm long per unionid and all mussels  $\geq 11$  mm long. In total, 3000 mussels of lengths  $\leq 10$  mm and 585 mussels  $\geq 11$  mm long were measured from the August samples. Length-frequency distributions of unmeasured mussels  $\leq 10$  mm long were based on the proportions of the 3000 measured mussels  $\leq 10$  mm long in each whole millimeter size group. Age groups were based on the lengths corresponding to the smallest number of mussels between length-frequency distribution peaks and the known time zebra mussels were in the canals as determined by annual spring and fall SCUBA diver inspections since 1987. Age designations (e.g., young-of-the-year, one-year-old, etc.) were determined based on a January 1 birthday. Dry weights (105°C for 48 h) of individual unionids and infesting mussels were determined for samples collected in August. Dry weights were determined for mussels 1 to 10 mm long as a group and for mussels 11 to 38 mm long as individual whole millimeter intervals. Infestation is used to describe

the colonization of zebra mussels on unionids. This follows the concept that mussels are ectoparasites as described by Margolis et al. (1982). Unionid taxonomy follows Turgeon et al. (1988).

## RESULTS

A total of 67,992 zebra mussels were removed and counted from 19 live unionid mollusks (Table 1). Unionids ranged between 83 and 142 mm and zebra mussels between  $<1$  and 38 mm in length. In general, unionid species and size composition were similar between sampling periods. In February, the intensity of zebra mussel infestation was low with a mean of 24/unionid and a range between 7 and 48/unionid (Figure 2). In August, the intensity of infestation increased to a mean of 6,777/unionid and a range between 2,491 and 10,732/unionid. The three highest intensities were observed on individual specimens of *Anodonta grandis* in February and on *Leptodea fragilis* and *Proptera alata* in August. No differences were observed between the length-frequency distributions of mussels on individual unionid bivalves.

Increases in infestation intensity of unionids by zebra mussels between February and August is attributed to increased numbers of young-of-the-year (YOY), one-year-, and two-year-old mussels (Figure 3). In February, one-year-old mussels ( $n = 25$ ; 2 to 6 mm in length) comprised about 22% and two-year-old mussels ( $n = 90$ ; 9 to 21 mm in length) comprised about 78% of the infesting population. Between February and August, YOY mussels ( $n = 67,106$ ) entered the population and the mean number of one-year-old mussels increased by a factor of 15 ( $n = 407$ ) and the number of two-year-old mussels increased by a factor of 2 ( $n = 178$ ). In August, YOY (1 to 12 mm in length) comprised 99.1%, one-year-old mussels (13 to 23 mm) 0.6%, and two-year-old mussels (24 to 38 mm in length) 0.3% of the population. The percent of YOY mussels by length in August was 1 mm = 24, 2 mm = 26, 3 mm = 17, 4 mm = 14, 5 mm = 9, 6 mm = 5, 7 mm = 2, 8 mm = 1, and 9 to 12 mm mussels composed about 1% of the total mussel infestation.

In August, mean dry weight of infesting zebra mussels was equal to 74% of the mean unionid weight (Table 2). Dry weights of unionids ranged between 12.6 and 119.2 g/unionid, and weights of infesting mussels ranged between 30.0 and 54.9 g/unionid.

TABLE 1.

Species and lengths of unionid mollusks, and total number of infesting zebra mussels, *Dreissena polymorpha*, collected from a power plant intake canal located in western Lake Erie, February 15 and August 10, 1989.

February 15			August 10		
Unionid		Number of Zebra Mussels	Unionid		Number of Zebra Mussels
Species	Length (mm)		Species	Length (mm)	
<i>Anodonta grandis</i>	97	30	<i>A. grandis</i>	100	5119
<i>A. grandis</i>	124	25	<i>A. grandis</i>	102	2491
<i>A. grandis</i>	128	30	<i>A. grandis</i>	111	5481
<i>A. grandis</i>	129	24	<i>A. grandis</i>	124	6841
<i>A. grandis</i>	130	48	<i>A. grandis</i>	127	6227
<i>Leptodea fragilis</i>	85	7	<i>A. grandis</i>	134	7759
<i>L. fragilis</i>	120	18	<i>A. grandis</i>	142	4426
<i>L. fragilis</i>	126	13	<i>L. fragilis</i>	83	9959
<i>L. fragilis</i>	135	25	<i>L. fragilis</i>	113	10732
			<i>Proptera alata</i>	105	8737
Mean $\pm$ S.E.	119 $\pm$ 5.6	24 $\pm$ 3.9		114 $\pm$ 5.6	6777 $\pm$ 811.1

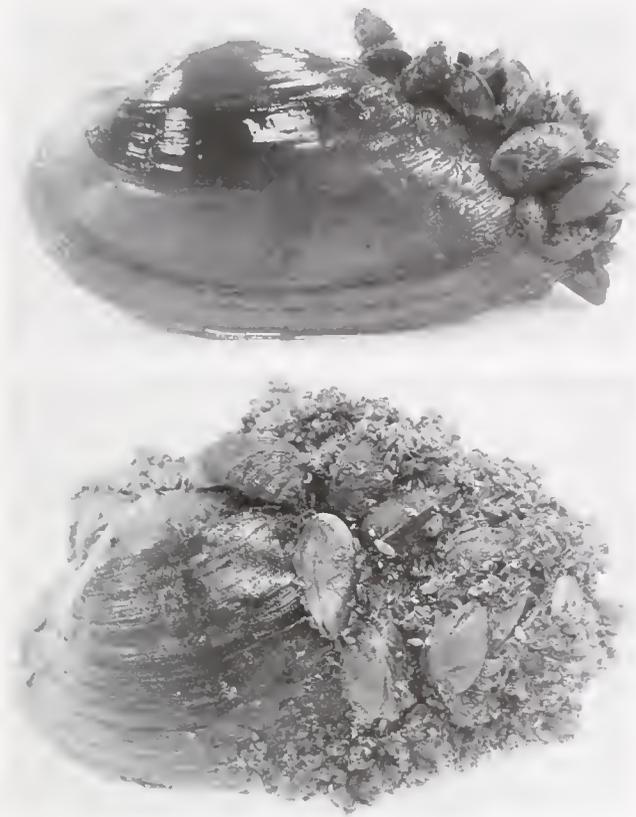


Figure 2. Unionid mollusks, *Anodonta grandis*, and infesting zebra mussels collected from a power plant intake canal located in western Lake Erie February 15 (top) and August 10 (bottom), 1989. Infestations are typical of that found before (February) and after (August) mussel spawning in summer 1989.

Weights of mussels ranged between 46 and 379% of individual unionid weights. Mean dry weights of YOY (15.4 g/unionid), one-year-old (13.4 g/unionid), and two-year-old (16.0 g/unionid) mussels each accounted for about one-third of the infesting mussel biomass. One unionid (12.6 g) was infested by a relatively low proportion (89 compared to 141 and 149%) of one-year-old mussels and another (38.8 g) by a high proportion (51 vs 25 and 24%) of YOY mussels. Five unionids were found to have infestations that weighed about the same or more than the host bivalve. These five were composed of three *Anodonta grandis* (87 to 121% of host clam) and two *Leptodea fragilis* (99 to 379% of host).

#### DISCUSSION

Infestation intensities of unionids by zebra mussels in February were similar to rates found in other studies, whereas in August, intensities were much greater than in previous studies except in Lake St. Clair in 1989 (Hebert et al. 1991). Maximum intensity in February (48/unionid) was similar to that (38/unionid) found by Hebert et al. (1989) in 1988 in Lake St. Clair, immediately upstream from Lake Erie. By 1989, Hebert et al. (1991) found similar densities of zebra mussels infesting unionids in Lake St. Clair as those found in the present study (i.e., in excess of 10,000 mussels/unionids). In Europe, infestation rates of unionids, as reviewed by Lewandowski (1976), were usually less than 50/unionid with a maximum intensity of 186 mussels/unionid. The August

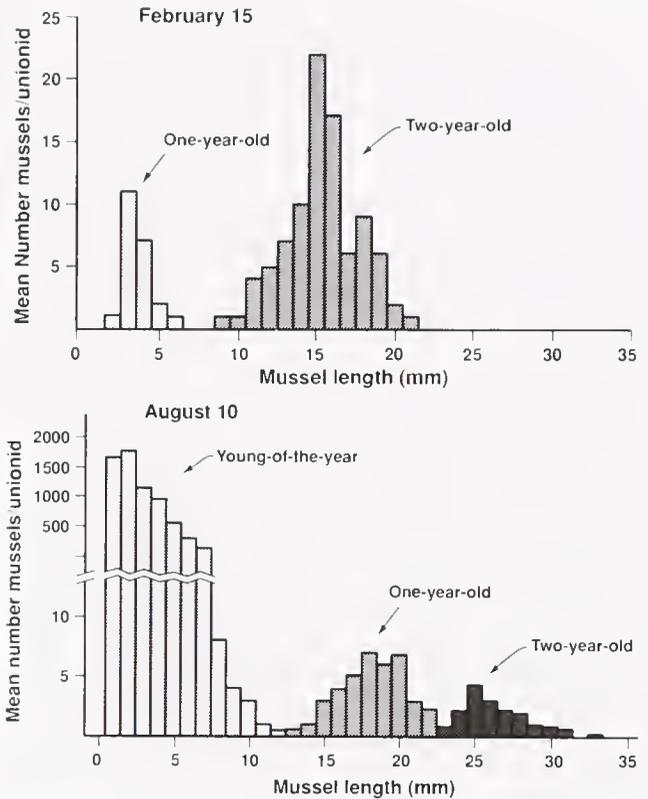


Figure 3. Length–frequency distribution of zebra mussels infesting unionid mollusks in a power plant intake canal in western Lake Erie, February 15 and August 10, 1989.

collections revealed a maximum intensity of 10,732 mussels on one unionid.

The high infestation intensities of zebra mussels on native unionids were attributed to large numbers of young-of-the-year mussels spawned in the summer of 1989 and the ability of mussels to attach to unionids and existing zebra mussels. Unionids do not provide enough exposed substrate to support 10,000 zebra mussels. The ability of zebra mussels to grow attached to other zebra mussels results in large mussels creating habitat for others to colonize in areas where suitable habitat is not present (Korschelt 1891). In Mikolajskie Lake in Poland, Lewandowski (1976) determined that less than 1% of infesting zebra mussels on unionids were less than 5 mm in length between May and September; whereas in August of the present study, greater than 95% of the mussels were less than 5 mm long. The piling of mussels on mussels may have negative effects on zebra mussels in the lower layers that are deprived of food and other environmental requirements. However, no dead zebra mussels were observed in this study.

Increased mean intensities of infesting one-year- and two-year-old zebra mussels between February and August indicate that mussels moved from surrounding substrates onto unionid shells. Zebra mussels are capable of active movement for at least the first year of life and possibly longer (Morton 1969, Mackie et al. 1989). One-year-old mussels increased on unionids by a factor of seven to eight times more than the increase observed for two-year-old mussels. Observations in natural waters in Europe indicate that unionids are the preferred substrate for zebra mussels (Biryukov et al. 1964, Morton 1969). This has been confirmed in laboratory studies (Lewandowski 1976), where infestation of live unionids is

TABLE 2.

Dry weights (g) of unionid mollusks and infesting zebra mussels in western Lake Erie August 10, 1989. Numbers in parentheses are percent dry weight of mussels (i.e., g mussels/g unionid  $\times$  100).

Unionid	Zebra Mussels			
	Total	Young-of-the-year	One-year-old	Two-year-old
36.6	44.5 (121)	11.2 (30)	16.3 (46)	17.0 (45)
34.4	30.0 (87)	9.2 (27)	12.9 (37)	7.9 (23)
52.1	45.2 (87)	13.2 (26)	16.1 (31)	15.9 (30)
70.3	44.8 (64)	19.0 (28)	13.6 (19)	12.2 (17)
96.4	53.3 (55)	20.4 (21)	14.5 (15)	18.4 (19)
83.7	45.7 (55)	13.3 (16)	13.5 (16)	19.0 (23)
119.2	54.9 (46)	11.4 (10)	14.6 (12)	28.9 (24)
12.6	47.8 (379)	17.8 (141)	11.2 (89)	18.8 (149)
38.8	38.6 (99)	20.0 (51)	9.6 (25)	9.0 (24)
61.8	43.0 (70)	18.5 (30)	11.8 (19)	12.8 (21)
x = 60.6	44.8 (74)	15.4 (25)	13.4 (22)	16.0 (27)
Range = 34.4–119.2	30.0–54.9 (46–379)	9.2–20.4 (10–141)	9.6–16.3 (12–89)	7.9–28.9 (17–149)

preferred over stones and dead unionids, indicating that zebra mussels find some advantage to infesting larger unionids.

The high total weight of infesting zebra mussels on unionid hosts has rarely been previously documented. Hebert et al. (1991) reported that at sites in Lake St. Clair with the highest densities of zebra mussels, the weight of infesting mussels was as large as three times the weight of a unionid (maximum 250 g, wet weight?). The present study indicates that the weight of mussels/unionid is substantial and may exceed host weight by a factor of four (dry weight). As the largest proportion of the mussels continue to grow (i.e., 99.1% young-of-the-year), the weight of infesting mussels will continue to increase if survival of this age group is high. If the weight of mussels becomes too great for unionid locomotion, unionids may sink into the unconsolidated, muddy sediments found throughout much of western Lake Erie (Herdendorf and Braidech 1972). Covering of unionids with sediments cause most species of unionids to be smothered and starve. Even if they do not sink into the sediments completely, the metabolic costs of carrying zebra mussels may adversely affect unionids.

In Europe, colonization of unionid mollusks by zebra mussels has been observed (Korschelt 1891, Sebestyen 1938, Kuchina 1964, Lyakhov and Mikheev 1964, Zhadin 1965, Kornobis 1977) but little information about the possible positive or negative effects of zebra mussel infestation is available. Some negative effects of zebra mussel infestation on large unionids in Europe were documented by Wolff (1969), Lewandowski (1976) and Sebestyen (1938). Wolff (1969) observed that infestation resulted in com-

pletely deformed unionids, and Lewandowski (1976) documented deformed unionid shells for 8 of 336 infested unionids. In addition, Lewandowski (1976) reported that small unionid mollusks (*Anodonta piscinalis* Nilsson; 39 to 74 mm in length) with infestation intensities of about 40 mussels/unionid had smaller shell lengths than non-infested unionids. Sebestyen (1938) documented the invasion of zebra mussels into Lake Balaton, Hungary, and describes the effects of mussel infestation on unionids as follows: "There seems no doubt, that *Dreissenia* has a decided ill effect on Unionidae . . . From the very beginning *Dreissenia* settled in great numbers on the shells of these (unionids)." Sebestyen (1938) based her conclusion on observations and remains of infested unionids that accumulated on the shores of Lake Balaton. A comparison of the speed and magnitude of the zebra mussel invasion in Lake Balaton and western Lake Erie have been noted as being very similar (Schloesser 1990). Another biofouling mollusk, *Mytilaster* sp., has been observed to settle on shells of bivalve mollusks, attach with byssal threads, and suffocate the host (Zhadin 1965). To date, no positive effects of zebra mussel infestation on larger unionid bivalves have been determined.

Infesting zebra mussels are believed to negatively affect native unionids if they (1) impair normal locomotion and burrowing activities, thus preventing escape from environmental extremes, (2) prevent valve closure, thus exposing the unionid to predation, parasitism, and environmental extremes, (3) prevent valve opening, thus stopping respiration, (4) smother siphons, thus stopping metabolic activities, (5) eliminate food from the water reaching the unionid, thus causing starvation, (6) cause shell deformities, thus preventing normal growth, (7) generate metabolic wastes, thus causing toxic effects, and (8) add weight to the unionid shell, thus causing the host to sink into soft sediments where smothering would occur (In part, Mackie 1990). To date, our visual observations in Lake Erie suggest that zebra mussels have prevented valve closure, prevented valve opening, smothered siphons of native unionids, and possibly created anaerobic habitat conditions around host unionids.

At present, little is known about the possible negative effects of zebra mussel infestation on unionid mollusks in North America. Only a few of the possible mechanisms through which zebra mussels may affect native unionids have been observed in European waters (Lewandowski 1976). At low densities, such as those found in Europe (ca. 40–180/unionid, Lewandowski 1976) and the Great Lakes in 1988 (ca. 0–20/unionid, Hebert et al. 1989) zebra mussel infestation has not been shown to substantially affect populations of unionid mollusks. However, the effect of infestation at high intensities on unionids observed in Lake Erie in the summer of 1989 may lead to elimination of some or all unionid species. Infestation of native unionids in the nearshore waters of western Lake Erie is not likely to decrease in the future unless the number of zebra mussels in the area declines substantially.

The zebra mussel has expanded its geographical range in North America at a rate of 150 km/year between 1986 and 1990 (Griffiths et al. 1991). As the range of the mussel expands, it will overlap with the range of other unionid mollusks and may infest already stressed and endangered freshwater unionid mollusks in North America and Mexico (Turgeon et al. 1988). At present, we do not know if all unionid mollusks are threatened by the zebra mussel. The survival of endangered and other native mollusks may depend on the ultimate densities and range achieved by the zebra mussel invasion and the success of efforts to control them.

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## ENDOD IS LETHAL TO ZEBRA MUSSELS AND INHIBITS THEIR ATTACHMENT

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**ABSTRACT** The invasion of zebra mussels into the Laurentian Great Lakes has resulted in biofouling of water intakes of public utilities. Both static bioassays and assays using a recirculating-flow system with solutions of the African soap berry *Phytolacca dodecandra*, or Endod, were used to develop the experimental basis for mitigation and control methods. The molluscicides, Lemmatoxins, in Endod were lethal to zebra mussels at concentrations higher than 20 mg/L while lower concentrations inhibited attachment of adult mussels. Our results on the lethal effects of Endod, its inhibition of attachment, and its apparent biodegradability show Endod to be a candidate for controlling zebra mussel populations in restricted localities such as water intake pipes of water works.

**KEY WORDS:** Endod, molluscicides, focal mitigation, *Phytolacca dodecandra*, *Dreissena polymorpha*

### INTRODUCTION

The zebra mussel *Dreissena polymorpha* (Pallas), a native of Eastern Europe, has recently been introduced into the Great Lakes of North America, presumably through the dumping of ballast water from ocean going vessels. Since its discovery in 1988 in Lake St. Clair (Hebert et al. 1989) the mussels have been found in great quantities along lake shores and in water intakes of various water works, especially on Lake Erie. The veligers, larvae, are free swimming resulting in a wide distribution. After one to two weeks, the veligers are able to attach themselves to almost any solid surface and to each other, with their byssal threads, proteinaceous fibers with adhesive at the ends. Their ability to cluster into large aggregates, extensive proliferation and wide distribution, combined with the possible lack of natural predators in the Great Lakes may threaten fish spawning grounds as well as cause problematic fouling of utility pipes, screens etc. Existing chemical and mechanical methods are being used to control the population growth and at the same time the search for new methods has been intensified.

The experimental approach taken in this investigation is in the context of controlling zebra mussels by cost effective methods and simple applications using natural substances. Pilot studies using solutions prepared directly from powdered *Phytolacca dodecandra*, commonly known as Endod, to control populations of the snails (*Bulinus* and *Biomphalaria*) that are intermediate hosts for the protozoan causing schistosomiasis in some African countries (Mokhubu et al. 1987), indicate that Endod could also be used to control populations of *Dreissena*.

*P. dodecandra* is an indigenous plant in Africa whose berries have been used as soap for thousands of years. Lemma (1965,

1970) found that the berries possessed molluscicidal property, i.e., they were lethal to various species of snails that were intermediate hosts for the spread of schistosomiasis. Chemical studies of extracts of these berries revealed that the active molluscicidal compounds, called Lemmatoxins, were triterpenoid saponins (Parkhurst et al. 1973a and b. 1974). Using a bacterial system to assay mutagenicity and carcinogenicity, it was found that powder from dry berries was neither mutagenic nor carcinogenic (Lemma and Ames 1975). A solution of Endod powder loses its molluscicidal potency in two to three days presumably due to microbial degradation or modification in streams (Lemma 1970, Lemma and Yau 1974).

Laboratory experiments described in this paper demonstrate that simple preparations from Endod powder (without removing cellular debris and other biological materials) are effective toward zebra mussels. Together with results on the non-toxic nature of Lemmatoxins to mammals (Lambert et al. 1991), Endod appears to be a potential candidate as a mitigation agent in the control of zebra mussel populations.

### MATERIALS AND METHODS

Endod powder of approximately 250  $\mu\text{m}$  particle size of *P. dodecandra* (type 44) was obtained from the Institute of Pathobiology, Addis Ababa University, Ethiopia. Stock solutions of 1000 mg/L were prepared by dissolving the powder in aged and aerated tap water (referred as water unless otherwise stated). Insoluble cellular debris was not removed. Working solutions therefore also contained insoluble materials. Endod-S, a reference standard, was obtained from the Department of Pharmaceutical Technology and Biopharmaceutics of the State University Groningen, The Netherlands. Endod-S is hot-air dried powder prepared from the soluble fraction of the type-44 Endod berries, therefore containing no insoluble materials. Since both solutions of the raw powder and

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the Endod-S powder were not filtered nor clarified, they both contain cellular components that are essential for the synthesis of the Lemmatoxins (Parkhurst et al. 1973a and b). Freshly made Endod solutions are not active unless they have been incubated at 37°C for one hour or at room temperature for 16 hours. Longer incubation will lead to reduced molluscicidal potency, presumably due to microbial activity (Lemma 1970, Lemma and Yau 1974). Endod stock solutions used in this study were incubated in either manner and stored at 4°C until used. Although no potency tests were done, stock solutions older than one month were not used.

To test biodegradability or modification of the molluscicidal activity of Endod, solutions of 25 mg/L were filter-sterilized through 0.45 mm millipore filters and were stored in sterilized bottles for three days at room temperature before being used. Controls were not filtered.

Adult zebra mussels were collected between June and September 1990 at the shore of Lake Erie near the Toledo and Oregon pump stations, where there is no public access or industrial activity. The animals were scraped off the rocks and brought to the laboratory. The animals were then rinsed with water and placed in half-gallon fish bowls which were immersed into an 80-gallon recirculating aquarium (Living Stream, Frigid Unit, Inc. Toledo, Ohio) at 10°C. They were fed every other day with the unicellular alga *Clamydomonas*. Dead animals were removed daily. It was estimated that at least 70% have survived.

Prior to using the mussels in experiments, the fish bowls containing the animals were placed at room temperature with aeration overnight. For all bioassays, the animals were separated from their clusters. Unless stated otherwise, the mussels were placed in the appropriate vessels and allowed to acclimate for at least 24 hours before the addition of Endod. Animals of approximately 1.0–1.5 cm length were used. All experiments were done in water and some in lake water as indicated. Unless otherwise indicated, Endod containing water was replaced with clean water at 24 hours after treatment. Animals were not fed during exposures to Endod, but were fed afterwards.

To assay the effect of Endod in a static system, animals were placed in beakers, either 250 or 400 ml, depending on the number of animals used. The solutions were aerated.

A diagram of the recirculating-flow system is presented in Fig. 1. The pump was a Minipus-II (Gibson, Inc.) with a flow rate of 1.3 L/hr which was the sum of all four lines. 150 ml or 100 ml of

Endod solution at 1000 mg/L was delivered via one of these four lines. Detailed mathematical analyses of the flow dynamics, the precise Endod concentrations as a function of time and their relationship to the biological effects of Endod will be presented elsewhere.

Animals were considered dead when they failed to close their shells upon mechanical stimulation.

A probit analysis was performed to determine the LC<sub>50</sub> and LC<sub>90</sub> values and their 95% confidence limits for the experiment in which zebra mussels were exposed to Endod. The analysis was done with the SAS/STAT PROBIT computer program using SAS Version 5.18 (SAS Institute, Cary, NC) on <sup>10</sup>log-transformed Endod concentrations.

## RESULTS AND DISCUSSIONS

In one static bioassay done at room temperatures, the animals were not acclimated prior to the addition of Endod, i.e., Endod was added at the same time that the individual animals were placed in the beakers. The results of this experiment are shown in Table 1. Even after only 7 hours of treatment, a clear dose-effect relationship is evident (Fig. 2). The 24-h LC<sub>90</sub> and LC<sub>50</sub> values (with 95% confidence limit) were 19 (14.5–33.9 mg/L) and 8.8 (6.4–11.0 mg/L), respectively. Even though the animals were transferred to clean water after 24 hours of treatment with Endod, mortality continued to occur. For example, mortalities at 54 hours after treatment with 6.25 and 12.5 mg/L Endod reached respectively 85 and 100%. Therefore, it appeared that there was an effective exposure time during which no death occurred but which resulted in subsequent mortality in the absence of Endod.

This hypothesis of an effective exposure time was tested with a time course experiment. The Endod solutions were replaced with clean water at intervals after the beginning of treatment. The results are shown in Table 2. It appears that treatment for 4–8 hours was needed to yield a significant effect. The existence of the effective exposure time indicates a latent physiological response of the zebra mussels to Endod. This response was not investigated in

TABLE 1.

Mortality in *Dreissena polymorpha* exposed to various concentrations of Endod solution. Endod solutions replaced with freshwater after 24 hours. Ten mussels per replicate.

Exposure Concentr. (mg/L)	Replicate	Mortality (%)			
		@ 7 h.	@ 24 h.	@ 30 h.	@ 54 h.
0	A*	0 (10)**	0 (10)	0 (10)	0 (10)
	B*	0 (8)	0 (10)	0 (10)	0 (10)
6.25	A	0 (0)	30 (2)	60 (0)	90 (0)
	B	0 (0)	40 (2)	70 (0)	80 (0)
12.5	A	20 (0)	50 (0)	80 (0)	100
	B	0 (0)	70 (0)	90 (0)	100
25	A	70 (0)	100		
	B	40 (0)	100		
50	A	60 (0)	100		
	B	70 (0)	100		
100	A	90 (0)	100		
	B	80 (0)	100		

\* Mussels clustered together.

\*\* Values in parentheses are number of mussels attached to the surface of the beaker.

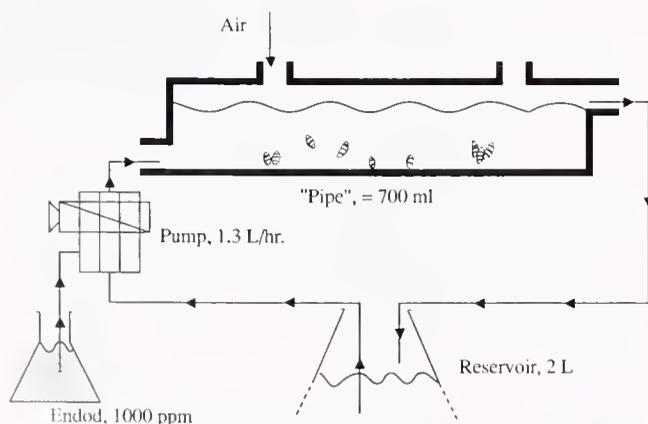


Figure 1. A recirculating system in which mussels were treated with Endod. Results of experiments using this system are shown in Tables 4 and 5.

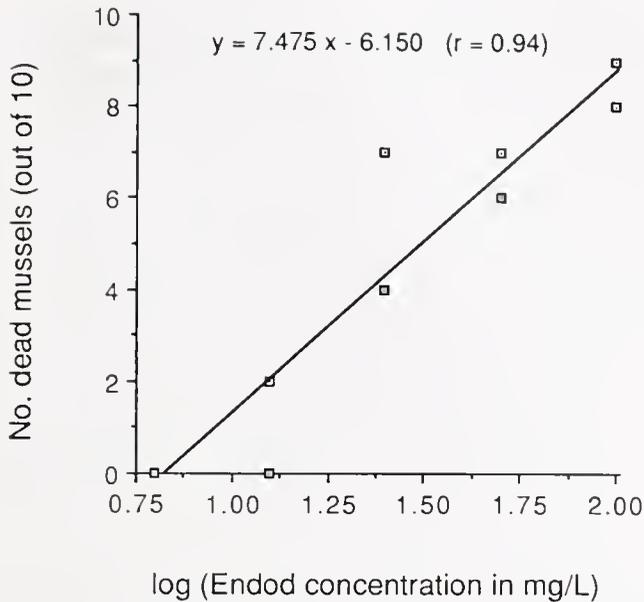


Figure 2. A semi-log plot of the mortalities in zebra mussels, 7 hours after being treated with Endod for 24 hours (data from Table 1).

the studies on the African snails. This result has significant implications in that prolonged and continuous applications may not be necessary to control mollusc populations.

In contrast to the exposure to the solution prepared directly from the powdered berries, in which 7 of the 20 animals were dead after 24 hours exposure to 6.25 mg/L (Table 1), a 22 hour exposure to Endod-S solution at the same concentration resulted in a mortality of 19 of 20 mussels (Table 3). Endod-S is more potent than the solution prepared from dry powder in this laboratory. Parkhurst et al. (1974) demonstrated that Lemmatoxins were present in the soluble fraction (the cytosol). Since Endod-S contains no insoluble materials, the concentrations of Lemmatoxins in the Endod-S are therefore expected to be proportionally higher than in the powder of the whole berries. Methods of isolation and purification of Lemmatoxins have been achieved (Parkhurst et al. 1973a, 1974) and the procedures have been patented (U.S.A. #3,813,383 and 3,886,272). The 24h-LC<sub>50</sub> for the toxicity of a butanol extract of Endod to the snail *Biomphalaria glabrata* is less than 3 mg/L (Stobaueus et al. 1990). However, the cost of producing Endod-S or purifying Lemmatoxins for large scale applications may be prohibitively high. In spite of the lower effectiveness of the powder of dry berries, their use may be cost-effective when a

TABLE 2.

Mortality in *Dreissena polymorpha* at 24 hours after start of exposures of different durations, to 25 or 50 mg/L Endod solution.

Exposure Duration (hours)	Mortality (%)		Sample Size
	25 mg/L	50 mg/L	
1	0	0	10
2	0	0	10
4	0	0	10
8	70	40	10
24	65	100	20

TABLE 3.

Mortality in *Dreissena polymorpha* exposed to various concentrations of Endod-S. Endod-S solutions replaced with freshwater after 22 hours. Ten mussels per replicate.

Exposure Concentr. (mg/L)	Replicate	Mortality (%)		
		@ 22 h.	@ 72 h.	@ 96 h.
0	A	0	0	0
	B	0	0	0
3.125	A	0	0	0
	B	0	0	0
6.25	A	90	90	100
	B	100	100	
12.5	A	100		
	B	100		
25	A	100		
	B	100		
50	A	100		
	B	100		

large quantity is required for controlling zebra mussels in intake pipes.

In the static experiment described above, individual animals were placed in beakers at the start of the experiments. In the controls, the animals were able to attach onto the pyrex glass surface and to cluster together within 12 hours. Some of the animals treated at 6.25 and 12.5 mg/L of Endod were not dead at 24 hours post-treatment, but most failed to attach (Table 1). The ability of Endod to inhibit the attachment and aggregation may or may not be related to the molluscicidal component. This observation has important implication for the control of zebra mussels in water intakes by the prevention of attachment of adults, and possibly post-veligers.

Prior to their exposure to Endod in the static bioassay, adult mussels were separated from the clusters and placed in beakers. This traumatic manipulation may have increased their sensitivity to Endod. Periodic tests using animals already attached to the beakers did not show significant differences from those reported here.

To study response of zebra mussels to Endod in a continuous flowing condition that water intake pipes operate, a recirculating-flow system was designed (Fig. 1). In this system the concentra-

TABLE 4.

Effects of Endod (150 ml) on zebra mussels in a recirculating system.

	Hours of Endod Treatment			
	2	3	4	6
Percent Mortality	34	50	53	82
Total # Animals	59	50	65	60
Attach Cluster	firm loose	weak very loose	weak very loose	none dispersed

Endod at 1000 mg/L was delivered via one of the four tubes (Fig. 1). Water in the reservoir was changed at the times indicated (after start of Endod). Mortality was recorded at 24 hours.

**TABLE 5.**  
Effects of Endod (100 ml) on zebra mussels in a recirculating system.

Hours Post Treatment	Percent Mortality				
	Hours of Endod Treatment				
	0.5	1	2	4	8
24 Hrs.	0	0	0	2	14
48 Hrs.	0	0	0	2	46
72 Hrs.	0	0	0	6	52
96 Hrs.	0	2	0	14	54
120 Hrs.	0	2	0	18	62

Endod at 100 mg/L was delivered via one of the four tubes (Fig. 1). Water in the reservoir was changed at the times indicated (after start of Endod). Fifty animals were used in each treatment. Mortality was recorded at 24 hour intervals after initiation of Endod treatment. Attachment and clustering were markedly reduced (none to weak, 0 to <35%) in the eight hour treatment.

tion of Endod in these experiments was calculated to be approximately 140 mg/L after 30 minutes when a total of 150 ml was delivered, assuming that at this point a negligible amount of Endod had entered the reservoir. It took 30 minutes to deliver 150 ml and approximately 60 minutes to reach equilibrium at about 50 mg/L in the system (pipe and reservoir). The water in the reservoir was changed to clean water directly after the exposures (lasting 2 to 6 hours), resulting in an equilibrium concentration of about 15 mg/L. After another change of the water 4 hours later, the remaining Endod concentration was about 4 mg/L. Mortality increased as the duration of exposure to Endod increased (Table 4). Since the animals had established their attachment prior to the treatment with Endod, the data of this experiment show that Endod treatment resulted in a loss of firm adhesion, and in some cases (last column, Table 4) dispersion from the clusters.

While the experiment described above used 150 ml of Endod stock, the second experiment used 100 ml of 1000 mg/L Endod in the recirculating-flow system. In this experiment the Endod concentrations were approximately two-thirds of the concentrations mentioned above. In this experiment, the animals were transferred to beakers with clean water at 24 hours after the beginning of the treatment. Mortality was determined daily. Although mortality was lower than in the first experiment in the same recirculating

system, a latent effect was observed similar to the static bioassays (Table 5). Virtually no mortality was observed when the animals were exposed to Endod for 0.5, 1, or 2 hours. The 4 and 8 hour treatment however clearly show the latent physiological response of zebra mussels to Endod; mortalities in Endod-free water increased 4 to 6 fold between 24 and 120 hours after the treatment.

Although the flow rate in our recirculating system is not the same as in water intakes, results did indicate that Endod is effective in a flowing system. The effective dose in this system appeared similar to that obtained in the static bioassay. A detailed analysis of the flow dynamics and its relationship to biological effects, which may be fundamental in designing a delivery system for Endod, will be presented elsewhere.

Earlier observations in field studies in Ethiopia suggested that Endod might be biodegradable (Lemma and Yau 1974). It was attributed to microbes present in the water and on the Endod berries. This preliminary observation was confirmed in the present study (Table 6). When microbes were filtered out from the 25 mg/L Endod solutions, Endod's molluscicidal potency was retained even after a 72 hour storage at room temperature. Storage of the non-filtered preparations at room temperature caused a reduced effect on zebra mussels, especially for sample #1. However, there appeared to be differences among preparations. Freshly prepared Endod was effective in all three preparations, with a weakest activity in the sample that was prepared in lake water (sample #3). The difference among the preparations may be attributable to differences in chemical compositions of the water samples. Future studies should include water chemistry and its effects on Endod's efficacy. The molluscicidal effect could therefore be modified biologically or chemically. Nevertheless, our results indicate that the reduction of the effectiveness of Endod when kept at room temperature is (at least partly) due to biodegradation, although no tests were done to compare microbial growth among the different preparations.

It is evident that the solutions of powder of dried Endod berries contains molluscicidal components which may be useful in the control of zebra mussel populations in certain restricted environments, such as water intake pipes. Recent safety evaluations of Endod-S were conducted in North American and European laboratories in accordance with the Minimal Data Requirements (Tier 1) and with the Guidelines for Pre-Market Chemicals of the Organization for Economic Cooperation in Development (Lambert et al. 1991). In Acute Mammalian Toxicity Tests, with the exception of the eye irritation toxicity test which indicated severe

**TABLE 6.**  
Effects of sterilization on the molluscicidal activity of Endod.

Hours Post Treatment	Percent Mortality											
	Controls						Treatments					
	Water			Endod			Non-Filtered			Filtered		
	1	2	3	1	2	3	1	2	3	1	2	3
24 Hours	0	0	0	10	10	0	0	0	0	10	10	0
48 Hours	0	0	0	95	35	10	0	10	0	85	35	5
72 Hours	0	0	0	95	70	20	0	45	0	85	75	5
96 Hours	15	0	0	100	80	35	5	75	0	85	75	25

Endod solutions at 25 mg/L in aged, aerated tap water (1,2) and lake water (3) were divided in half and processed as outlined in Materials and Methods. A freshly prepared Endod control (25 mg/L) and a water control were included in each set of assays. Twenty zebra mussels were used in each assay.

irritation, results were classified as either non-toxic or only slightly toxic. Ecotoxicity tests indicated that Endod is not any more toxic than synthetic molluscicides such as niclosamide (Monkiedje 1990). Because of these positively encouraging studies, Endod has been recommended for field trials in streams in Ethiopia and other African countries (Lambert et al. 1991). In addition to its molluscicidal activity, Endod also exhibits larvicidal (mosquitos), hirudinicidal, trematodicidal, spermicidal (human), and fungicidal properties (Lemma 1971). The molluscicidal component, the saponins, may or may not be responsible for the multiple actions of Endod. In addition, the biodegradability of Endod clearly indicates its potential for control of schistosomiasis as well as for focal control of *Dreissena*. This notion is further strengthened by the comprehensive study of Monkiedje (1990). This study indicated that Endod could be used in the environment because of results from US-EPA required tests including isotherm absorption to carbon and soil in accordance with Freundlich parameters. New

analytical procedures (thin layer chromatography and hemolytical assays with red blood cells) have been worked out to determine concentrations of Endod (Monkiedje 1990, Monkiedje et al. 1990). Monocultivation of Endod type-44 has been successful in Ethiopia and other African nations. The berries of type-44 have the highest molluscicidal saponin content among about 600 varieties assayed, approximately 25% by weight (Monkiedje 1990). That large scale preparations of Endod powder can be available on demand (Mokhubu et al. 1987) suggests that the use of Endod may be a cost-effective means for zebra mussel mitigation.

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## METHODS FOR EVALUATING ZEBRA MUSSEL CONTROL PRODUCTS IN LABORATORY AND FIELD STUDIES

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**ABSTRACT** The zebra mussel, *Dreissena polymorpha*, is causing great economic hardship among industries located on Lake Erie because of its ability to colonize water intakes and thereby impede water flow. The zebra mussel has also impacted commercial fishing operations by filtering massive quantities of phytoplankton which would otherwise be available to support the growth of game fish. Currently there is great interest in devising methods to control the mussel in Lake Erie and in designing control programs to address a spectrum of diverse needs.

Critical to providing the information to support control programs is the generation of data that are of high quality and which were derived through the use of standardized methods and are thus comparable. In addition, methods must be available to screen candidate control agents in the laboratory as well as the field.

We report herein methods for measuring the acute toxicity of molluscicides to zebra mussel adults. In addition, a protocol for assessing efficacy of antibiofouling paints in the field is presented. Using these methods, a variety of molluscicidal and molluscistatic products were evaluated for their activity against zebra mussels. In general, the data indicate that there is a potentially large number of chemicals which will kill or repel the zebra mussel.

**KEY WORDS:** zebra mussel, *Dreissena*, molluscicide, molluscistat, standard protocols

### INTRODUCTION

The zebra mussel, *Dreissena polymorpha*, is a nonindigenous bivalve whose biofouling activity is causing profound economic hardship in the Great Lakes. Since the mussel was first introduced in North America, ostensibly in the ballast water from an ocean-going vessel (Herbert et al. 1989, Mackie et al. 1989), the mussel has spread to all of the Great Lakes (Roberts 1990) and has reached densities in Lake Erie in excess of 70,000 per square meter (McMahon and Tsou 1990). The mussel actively colonizes pipes and other hard substrates by secreting byssal threads with which it attaches to the surface. Frequently, the zebra mussels accumulate in masses sufficient to reduce water flow to critically low levels (McMahon et al. 1990). In addition, an individual mussel has the ability to filter a volume of one liter of water per day. In so doing, the mussels collectively remove huge amounts of phytoplankton from the water thus reducing zooplankton abundance and depriving juvenile fish of an important food source. The U.S. Fish and Wildlife Service has estimated that 3.7 billion dollars in commercial fishing revenues will be lost due to the zebra mussel (Mackie et al. 1989).

The severity of the zebra mussel problem has prompted considerable interest in devising methods to control it. At present, a variety of chemical control agents which include surfactants (McMahon and Tsou 1990), heavy metals (Dudnikov and Mikheev 1968), chlorine (Jenner 1984, Morton 1969), ozone (Jenner and Janssen-Mommen 1989) and ammonium nitrate (Shetsova et al. 1978) are being considered as zebra mussel control agents. While all methods are potentially important, it is readily apparent that no single method will satisfy the diverse array of needs for zebra

mussel control. Approaches to controlling the zebra mussel must mirror the diversity of settings in which the mussels can be found. In all cases, standardized methods must be available to provide a systematic approach to screening candidate chemicals for a variety of potential uses.

Of the various control methods available, two general approaches to chemical control appear viable. The first is the use of molluscicides to kill the mussels. A diverse arsenal of molluscicides has been developed in the Soviet Union and Europe in an effort to control zebra mussels (McMahon and Tsou 1990). In North America, compounds developed to control the biofouling Asiatic clam, *Corbicula fluminea* may also be effective against zebra mussels (McMahon and Tsou 1990). The second chemical control strategy is to develop molluscistatic products which do not kill the mussel but deter attachment. Chemicals which are irritating enough to deter attachment may be useful in preventing secondary infestation once the primary infestation has been removed. Importantly, both molluscicidal and molluscistatic chemicals could be incorporated into paints to protect solid supports from zebra mussel attachment.

We describe herein protocols for measuring the acute toxicity of molluscicides to adults which take into consideration the biology of the mussel. In addition, we describe methods for evaluating the efficacy of molluscicidal and molluscistatic antibiofouling paints. Using these methods, we have evaluated the effectiveness of a variety of chemicals against the zebra mussel in both laboratory and field settings.

### MATERIALS AND METHODS

#### *Measurement of Acute Toxicity in Adult Dreissena*

Adult zebra mussels were obtained by scuba divers from the bottom of Lake Erie. Adults were maintained in Lake Erie water

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with oxygenation during transport back to the laboratory. In the lab, adult *Dreissena* were maintained in 55 gallon aquaria filled with aged tap water from which chlorine had dissipated. Approximately 2,000–3,000 mussels were maintained in each aquarium under full aeration at 20°C. Cultures were maintained for three–four months by feeding the colony every other day a slurry of pelleted *Chlorella* suspended in 100 mL of water which was then frozen. Frozen *Chlorella* cubes were suspended over the aquaria; the water containing the alga dripped slowly into the water as the cube melted; *Chlorella* was removed from water by the filtering action of the mussels. The water in which the mussels were housed was checked daily for accumulation of ammonia and was replaced completely every 2 days to prevent ammonia intoxication. Culture water was removed from the aquaria and treated with 3–5 mL 1 N HCl to kill any veligers that may have been produced prior to disposal.

Static acute toxicity tests with *Dreissena* adults were initiated by putting 500 mL of aged tap water, pH 6.0 into 1 L beakers. Thereafter, the water in each beaker received 1 mL of a given concentration of test chemical dissolved in acetone (or appropriate solvent); controls received 1 mL of solvent; three replicates of each concentration were made. Range finding tests were conducted initially to identify 5 concentrations projected to give 5–95% mortality.

Twenty-four hours prior to use in toxicity tests, adult zebra mussels, approximately 1.5–2.0 cm in size were removed from the stock culture by cutting their byssal threads with a razor blade. Groups of 12–13 mussels were then placed on the bottom half of 9 cm diameter glass petri dishes; the latter were immersed in fresh tap water from which chlorine gas had been allowed to dissipate. Mussels were allowed to reattach themselves to the petri dishes over a 24 hour period. Those mussels which reattached to the petri dish were considered healthy and acceptable for use in toxicity tests. Mussels which did not attach to petri dishes within 24 hours were discarded. A minimum of 10 adult zebra mussels was used for each replicate. Into each of the previously prepared test beakers, a petri dish containing attached zebra mussels was submerged. Test beakers were then placed in a Forma Scientific (Marietta, OH) #37422 environmental chamber set at the appropriate temperature on a photoperiod of 14:10 hours. In most cases, a standard temperature of 24°C was used. Selection of the mussels as test subjects as well as the position of the test beakers in the environmental chamber were randomly selected and placed into the environmental chamber.

Mortality was assessed every 24 hours for the duration of the toxicity tests. The criterion for mortality was failure to respond to the touch of a probe. In most cases, dead mussels gaped open. However, mussels with closed shells were examined by inserting the probe between the two shells just above the incurrent siphon. Mussels which showed no adductor activity were considered dead. The test was invalidated if control mortality exceeded 20%. The duration of the toxicity tests ranged from 24–96 hours.

The water in all beakers containing zebra mussels was adjusted to pH 1.0 with 0.5 mL 1 N HCl following the cessation of the test. Beakers were held an additional 24 hours to ensure that survivors of the toxicity test were killed by reduced pH before disposal.

To assure that test conditions were suitable for the mussels during the toxicity tests, DO, pH, total alkalinity, total hardness and temperature were monitored in controls and the highest concentration of toxicant at the beginning of each test and every 24 hr thereafter.

For all toxicity tests, data were analyzed using Finney's (1971) probit analysis to estimate LC<sub>50</sub> values and 95% confidence limits. Mortalities of 0 or 100% were not used in probit analyses; in every case, at least 3 partial kill points were obtained. LC<sub>50</sub> values were considered to be significantly different where 95% confidence limits did not overlap.

#### *Field Tests of Antibiofouling Paints and Substrates*

To test the effectiveness of a variety of chemicals in preventing the attachment of the zebra mussels, 20 cm by 20 cm fiberglass panels were coated with a given antibiofouling product according to manufacturer's instructions. For each test, 9 treatment panels and 3 unpainted control panels were assembled. Thereafter, groups of 3 panels (either treatment or control) were attached at one end to a 1 m wooden dowel rod using 50 lb fishing line. At the opposite end, the panels were attached using 50 lb fishing line to a 1 m metal tube which was filled with dry Quikcrete® cement. The assembly was then suspended in Lake Erie with the dowel rod at the water surface to mark the position of the submerged panels and the cement-filled metal tube as an anchor. The lengths of fishing line were adjusted so that the panels were suspended equidistance from the dowel rod and the metal tube anchor. In general, panels were anchored near Franz Theodore Stone Laboratory at Put-in-Bay on Lake Erie at a depth of 4–5 feet. At this depth, the panels were in contact with aquatic plants that had very high densities of adult zebra mussels.

The panels remained in the lake for a 3 month period and were withdrawn from the lake every 10–14 days to determine whether any adult zebra mussels had attached. After the number of adults attached to control and treated panels had been scored, the panels were resuspended in Lake Erie. The data were expressed as the number of adult zebra mussels attached to control and treated panels. Statistical differences between treatments and controls were analyzed using analysis of variance (ANOVA) (SAS 1982); means were separated using Duncan's (1951) Multiple Range Test.

#### *Chemicals*

The chemical control agents, both molluscicidal and molluscistatic, which have been evaluated are listed in Table 1. Also provided is information on chemical purity, formulation, source and type of test performed.

## RESULTS

#### *Acute Toxicity Tests*

The zebra mussel is susceptible to the toxic action of chemicals belonging to diverse chemical classes. The toxicity of Buckman 6002, a polyquaternary ammonium derivative, was measured both as a function of concentration and time. A dose-time-response was clearly evident; the LC<sub>50</sub> for Buckman 6002 declined from 1670 µg/L at 24 hr to 830 µg/L at 96 hr (Table 2). The molluscicide, 2-(thiocyanomethylthio) benzothiazole (Buckman 6009) proved to be even more toxic to adult zebra mussels with acute toxicity values ranging from 794 µg/L at 24 hr to 653 µg/L at 96 hr.

The effect of temperature on toxicity was measured using Calgon H-130 in acute toxicity tests with zebra mussel adults (Table 3). An increase in temperature from 24°C to 28°C generally lead to an increase in the toxicity of H-130 although the differences were not always statistically different. For instance, the marked de-

TABLE 1.  
Compounds used in laboratory and field tests against *Dreissena polymorpha*.

Source	Product	Active Ingredient	Test Type
Calgon	H-130	didecyl dimethyl ammonium chloride	acute toxicity
Fermenta	chlorothalonil	tetrachloroisophthalonitrile	acute toxicity, field
Philadelphia Resins	TBT	tributyltin methacrylate	field
Philadelphia Resins	cuprous oxide	cuprous oxide	field
Buckman	6002	poly[oxyethylene-(dimethyl-iminio)ethylene-(dimethyl-iminio)ethylene dichloride	acute toxicity
Buckman	6009	2-(thiocyanomethylthio)] benzothiazole	acute toxicity

crease in the 96 hr LC<sub>50</sub> from 697 µg/L at 24°C to 275 µg/L at 28°C was not statistically different due to the large 95% confidence limits reported for the 24°C, 96 hr LC<sub>50</sub>. However, the statistically significant increases in toxicity between temperatures at 48 and 72 hr clearly conveyed the effect of increasing temperature on toxicity. As with Buckman 6002, the toxicity of Calgon H-130 was seen to be both concentration and time-dependent (Table 3).

#### Field Tests with Antibiofouling Paints

Chlorothalonil was applied as an antibiofouling paint in three different formulations denoted 1, 2 and 3, respectively. All contained up to 19.5% chlorothalonil as the active ingredient. Within one hour of being set out into Lake Erie, 3 adult zebra mussels were detected on the surface of a single panel treated with formulation 2. However, the mussels did not secrete a byssal thread attachment and were gone by the time the panels were next inspected. No other adult zebra mussels were detected on the panels treated with any formulation of chlorothalonil throughout the 3 month test period (Table 4). Among control panels, the number of adult mussels attached varied from a low of 0 for formulation 3 on August 27 to a high of 75 adults on formulation 3 on October 22. The number of mussels attached to control panels varied considerably over time. In some cases, mussels were seen to attach and then later detach leaving remnants of the byssal threads behind as evidence of the mussel having been present. The data entries in Tables 4 and 5 thus reflect only adult mussels present on a particular date and not cumulative attachment over time.

The two heavy metals (TBT and cuprous oxide) were seen to be effective in deterring attachment of adult zebra mussels during the 3 month field season (Table 5). No adult zebra mussels were detected on any of the treated panels at any time during the field

season. However, adult mussels regularly attached to the control panels. As with the chlorothalonil control panels, adult zebra mussels attached to control panels and later detached, leaving behind byssal threads. It should be noted, that due to a shortage of materials only 3 treated panels of each type were used in these assays.

## DISCUSSION

#### Critique of Methods

Various components of the zebra mussels' biology are relevant to the assessment of acute toxicity and must therefore be accounted for in designing protocols for estimating LC<sub>50</sub>s. Of obvious importance are a variety of water quality parameters which include water type, oxygen content, pH and temperature.

The use of a reconstituted standard reference water, i.e. distilled, deionized water to which known concentrations of salts are added back would have the benefit of eliminating variability in important water quality parameters. However, the standard reference water prescribed by USEPA (1975) cannot be used since two of the major components, KH<sub>2</sub>PO<sub>4</sub> and KCl, are lethal to the zebra mussels at the recommended concentrations (Fisher et al. 1991). The mussels survive readily in aged tap water and distilled water. The former was chosen for use in these tests until a suitable reconstituted water can be found.

The survival of *Dreissena* in natural water is limited when oxygen concentrations fall below 7 ppm. Adult *Dreissena* are known to be sensitive to reductions in O<sub>2</sub> content (Mikheev 1968). Thus, O<sub>2</sub> content must be monitored during toxicity tests in order to assure validity of the results. Each treatment beaker was aerated during toxicity tests, to maintain oxygen levels compatible with

TABLE 2.  
Toxicity of molluscicides to zebra mussel adults.

Compound	Test Duration	LC <sub>50</sub> (µg/L)	95% C.L.
Chlorothalonil	24	730	680-777
Buckman 6002	24	1670	1360-2220
Buckman 6002	72	980	790-1030
Buckman 6002	96	830	760-910
Buckman 6009	24	794	683-867
Buckman 6009	48	679	494-756
Buckman 6009	72	672	467-744
Buckman 6009	96	653	398-733

TABLE 3.  
Toxicity of calgon H-130 to adult zebra mussels.

Temperature °C	Exposure Time (hr)	LC <sub>50</sub> * µg/L	95% C.L.
28	24	928 <sup>de</sup>	819-1082
28	48	596 <sup>c</sup>	521-667
28	72	413 <sup>b</sup>	354-469
28	96	275 <sup>a</sup>	217-323
24	24	1103 <sup>e</sup>	1008-1268
24	48	826 <sup>d</sup>	780-873
24	72	765 <sup>d</sup>	714-812
24	96	697 <sup>abcde</sup>	27-1025

\* LC<sub>50</sub> values followed by the same letter are not significantly different as determined by overlapping 95% C.L.

TABLE 4.

Field evaluation of chlorothalonil as an antifouling paint.

Panel Type Formulation	Number of Adult Mussels Attached*					
	8/9	8/27	9/10	9/17	10/2	10/22
Chlorothalonil 1	0	0	0	0	0	0
Control	0	41	10	19	13	0
Chlorothalonil 2	1	0	0	0	0	0
Control	0	9	14	21	0	5
Chlorothalonil 3	—	0	0	0	0	0
Control	—	—	40	33	16	75

\* Panels were set out in Lake Erie on 8/9/90 with the exception of formulation 3 which were set out on 8/27. Panels were read after 1 hr of submersion of 8/27 and then on the dates indicated.

zebra mussel survival. This procedure kept dissolved oxygen levels in the range of 7.4–10.8 ppm which was sufficient to prevent oxygen depletion from becoming a cause of death. However, it should be noted that dead mussels had to be removed from the beakers at least every 24 hours in order to avoid a reduction in oxygen concentration in response to decaying zebra mussels. Likewise, the pH of the water in which the tests were performed was important. Zebra mussels will tolerate a range of pHs which may be as broad as 5.0–8.5 (Fisher et al. 1991). However, control mortality began to increase when the pH level of the aged tap water was experimentally set below 5.0 while pH values in the range of 5.5–8.0 did not affect survival of control adults. The pH of aged tap water consistently fell in the range of 5.8 to 6.0 and this pH level was used throughout because it required no amendment with buffer and because the mussels readily tolerated this pH level.

Water temperature is a variable which is an extremely important arbiter of molluscicidal activity in *Dreissena*. An increase in temperature leads to an increase in metabolism and a concomitant increase in oxygen demand (Mikheev 1968). Zebra mussels are thus more sensitive to oxygen deprivation at higher temperatures than they are at lower ones. In the present study, oxygen levels are maintained at levels greater than 7.0 ppm even at higher temperatures. Thus, the potential interaction between oxygen and temperature was probably not responsible for changes in molluscicidal activity. However, there was a significant increase in the toxicity of Calgon H-130 with a temperature increase of 4°C (Table 3). This finding is consistent with other studies on adult *Dreissena* in which the toxicity of a variety of molluscicidal compounds increased with an increase in water temperature (Jenner 1984, Lyakhov 1968, Mikheev 1968). Because significant changes in tox-

TABLE 5.

Field Evaluations of TBT and cuprous oxide as antifouling paints.

Paint Type	Number of Adult Zebra Mussels Attached				
	8/9	9/10	9/17	10/2	10/22
TBT (12%)	0	0	0	0	0
Cuprous Oxide (42.4%)	0	0	0	0	0
Control	40	14	21	21	77

icity can occur over a narrow temperature range, temperature must be carefully controlled in toxicity determinations.

Two elements of adult zebra mussel behavior can have an important effect on the outcome of toxicity tests. The first of these is their filtering activity which can be continuous or intermittent. In addition, there is some evidence that zebra mussels will close their shells in response to the presence of chemicals or in response to unfavorable environmental conditions. This limits their exposure to the chemical and leads to variability in toxicity results (McMahon et al. 1990). The proposed test method minimizes this source of variability by maintaining a temperature consistent with optimal filtering activity (Mikheev 1968) and providing an acclimation period for the mussels to adapt to the test environment. The mussels were observed to be actively filtering 90–95 percent of the time when filtering activity was monitored every 30 min for 8 hr. The other important behavioral factor is the requirement for attachment of the adult mussels to a hard surface with byssal threads. It is disruptive to the mussel and potentially detrimental to cut the byssal threads in order to evaluate the condition of the mussels (McMahon et al. 1990). However, it was desirable to take multiple readings of the mussels' condition. The necessity of severing the byssal threads to take readings was eliminated by allowing the mussels to attach to a glass petri dish. The latter could be submerged and then retrieved affording an evaluation of the mussels' condition which did not require detachment.

The method employed for field testing of antifouling paints appeared to be successful (Tables 4, 5). Although the only panel type tested was fiberglass, other substrates such as concrete, wood or aluminum could have been substituted. The use of an adequate number of replicate panels was of paramount importance to this assay. There was sufficient variability in attachment of adults to control panels, both between replicates and at different time periods, that replication was essential to accuracy. In addition, the importance of taking multiple readings over an extended period of time was clearly demonstrated by the variability in attachment to control panels.

Since environmental conditions are extremely important to zebra mussel survival, the field assay could be improved by evaluating attachment of adults at different depths for which temperature, oxygen content and light quality will vary. Future evaluation of antifouling paints should also include a microscopic evaluation of veliger settling and attachment to control and treated panels.

#### Evaluation of Molluscicide Efficacy

A variety of structurally diverse chemicals were acutely toxic to adult zebra mussels (Tables 2, 3). All four molluscicides tested against adult *Dreissena* produced LC<sub>50</sub> values in the range of 275–1670 µg/L. Toxicity was responsive to concentration, time of exposure and temperature. As each of these variables increased, toxicity was likewise accentuated.

Adult zebra mussels proved to be much more sensitive to Buckman 6002 in the current study than is reported elsewhere. McMahon et al. (1990) measured LT<sub>50</sub> values for adult *Dreissena* exposed to Buckman 6002 at levels between 0.5–8 mg/L. At an exposure concentration of 2 mg/L, 50% mortality was achieved after approximately 200 hours of exposure. However, our findings indicate that 50% mortality will occur within 24 hours at a concentration of 1.6 mg/L (Table 2). The discrepancies in these findings may be attributed primarily to the fact that the tests conducted

by McMahon et al. (1990) were performed at 20°C as opposed to 24°C used in the present study. The ability of elevated temperature to increase toxicity is evident with Calgon H-130 (Table 3) and has also been reported for chlorine (Jenner 1984, Greenshields and Ridley 1957), heavy metals (Dudnikov and Mikheev 1968, Lukanin 1968) and ammonium nitrate (Shevtsova et al. 1978). The utility of combining a slightly elevated temperature with molluscicide treatment in contained settings such as pipes bears investigation.

Just as a variety of chemicals have proven efficacious in killing adult zebra mussels, so too are many chemicals effective in deterring attachment to solid surfaces. Heavy metals such as copper and tributyltin oxide (TBT) have been used widely as marine antifouling agents (Jenner and Janssen-Mommen 1989). These same chemicals appear to have application against the zebra mussel (Table 5). However, because heavy metals have in general and TBT in particular have been identified as significant pollutants with undesirable nontarget effects (Friberg et al. 1979), the finding that chlorothalonil prevents attachment of adult zebra mussels is important (Table 4). Although chlorothalonil is toxic to fish (Da-

vies and White 1985), its half-life in nonsterile aqueous systems containing sediments is less than 3 days (Walker et al. 1988). Chlorothalonil appears to be useful in several paint formulations and may prove to be effective in preventing zebra mussel attachment.

In conclusion, it is clear from these data that a variety of chemicals are effective in killing zebra mussels and preventing attachment. The procedures described herein should facilitate screening of a large number of candidate chemicals for determining efficacy in controlling *Dreissena* both in laboratory tests and in the field. This, in turn, will promote the accumulation of a body of data from which chemicals, having different merits, can be evaluated for use in zebra mussel control in a variety of different settings.

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## EXPERIMENTAL FISHING FOR THE FLYING SQUID, *OMMASTREPHEs BARTRAMI* (LESUEUR, 1821), OFF BRITISH COLUMBIA

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Canada V6N 3T5

**ABSTRACT** In July and August, 1983 approximately 329 t of the oceanic 'flying' squid, *Ommastrephes bartrami* (Lesueur), were taken in 40 drift-gillnet sets from 100 to 400 km off the British Columbia coast between 47°07' and 53°22' latitudes N. Drift-gillnets 8.5 m deep were set in waters with surface temperatures ranging from 13.9 to 16.3°C. Catch per unit effort increased when sampling across sea surface thermal discontinuities and was correlated with a 14 day cycle, likely associated with phases of the moon. An estimated 188.8 t of pomfret, *Brama japonica* Hilgendor, represented 72.4% of the by-catch of 19 fish species. By-catch of salmonids was <0.6% of estimated total catch. Jigging for flying squid on 41 nights yielded poor catches.

**KEY WORDS:** Oceanic squid, *Ommastrephes*, drift-gillnet, British Columbia

### INTRODUCTION

Recently, much has been published on the biology and distribution of north Pacific oceanic squid (Osako and Murata 1983, Jefferts 1986, Murata 1990), especially *Todarodes pacificus* Steenstrup, whose domination of the northwestern region's squid landings has been diminishing (Okutani 1983, Murata 1989). Due largely to expansion of Japanese exploratory fishing, northern Pacific oceanic squid in general have received more attention (Kubodera et al. 1983, Murata 1990) although the northeast Pacific remains relatively less explored (Sato and Hatanaka 1983). Rathjen (1983) reviewed North American squid fisheries. The only established Pacific fishery is for the relatively inshore *Loligo opalescens* Berry (Recksiek and Frey 1978, Bernard 1980) and there is little oceanic squid fishing (Jefferts 1986).

A Japanese jigging fishery for the oceanic "flying" squid, *Ommastrephes bartrami* (Lesueur), began in 1974 in the northwestern Pacific in response to decreasing landings of *T. pacificus*, and was followed in 1978 by a drift-gillnet fishery (Osako and Murata 1983). The Japanese now consider *O. bartrami* second in importance to *T. pacificus* in the northwestern Pacific squid fisheries (Araya 1983). Japanese exploratory drift-gillnetting for *O. bartrami* has been reported from east of 170°E longitude to 130°W (Araya 1983, Kubodera et al. 1983). Stocks of *O. bartrami* west of 170°E are declining because of over fishing (Araya 1983). Distribution and abundance of the species is monitored often (Murata and Shimazu 1982, Murata et al. 1983, Kobayashi et al. 1986, Nakamura 1988, Murata 1990).

Exploratory drift-gillnetting for *O. bartrami* off British Columbia has occurred in the summers of 1979, 1980, 1983, 1985, 1986 and 1987 (Bernard 1980, 1981, Robinson and Jamieson 1984, Sloan 1984, Jamieson and Heritage 1987, 1988). I report here on the distribution of *O. bartrami*, influences on catch per unit effort (CPUE) and by-catch in drift-gillnets off the British Columbia coast in the summer of 1983.

### MATERIALS AND METHODS

Between July 19 and August 31, 1983, 40 drift-gillnet sets were completed up to 400 km off the west coasts of Queen Charlotte and Vancouver Islands (Figure 1). Fishing was carried out by a 50 m Japanese vessel. After continuous monitoring of sea surface temperature, set locations were chosen between 14.0 to

16.0°C with a preference for closely situated isotherms. At the end of each sampling day the catch was censused in relation to position and temperature to enhance prediction of high squid abundance sites.

Drift-gillnets consisted of buoyed panels approximately 50 m long × 8.5 m deep, strung in lengths of either 6.25 or 12.5 km depending, respectively, upon poor or good weather conditions. Net groups had radio buoys on each end and drifted independently of each other. These net groups comprised the day's set. Green nylon monofilament netting with a stretch mesh of 118 to 121 mm was used.

A mean of 34.4 km (7.5 km SD; 12.5 to 43.2 km range) of net was deployed on a pre-determined course between approximately 1700 to 2130 h each fishing day. Inhauling began between 0400 to 0500 h the next day and required approximately 8 to 10 h. Position and sea surface temperature, which varied between 13.9 to 16.3°C, was recorded using a hull-mounted sensor at the start of deployment and inhauling of each net group. Nets were inhaled as quickly as possible because shark predation on the catch increases greatly in daylight hours (Bernard 1981). Squid and fish by-catch were removed by hand from the netting. A daily record was kept of the exact weight of squid and number of salmonids. The weight of all fish by-catch was estimated from mean weights and an approximation of numbers per net group. Sloan (1984) provides a detailed account of the fishing technique and the circumstances of each set.

From each of 34 of the sets, 30 *O. bartrami* were selected at random and their dorsal mantle length (DML) measured. Dorsal mantle length is a reliable convention used in squid studies (Araya 1983) and, in flying squid, has a close relationship with body weight, regardless of sex, over a wide size range (Ishii 1977). Some individuals were weighed but ship movement enabled approximations only.

Six of seven days in the first week of the cruise were spent jigging only. Jigging sites were generally too close to shore to permit drift-gillnetting (Fig. 1). Thereafter, on another 34 nights, jigging was carried out from the drifting vessel after gillnet deployment. Intense overhead illumination and automatic jigging machines, each with two lines of 25 lures were used (Sloan 1984). Jigging depth ranged between 45 to 60 m (mean = 52 m). On 6 nights hand-jigging was attempted using lines with pairs of large lures.

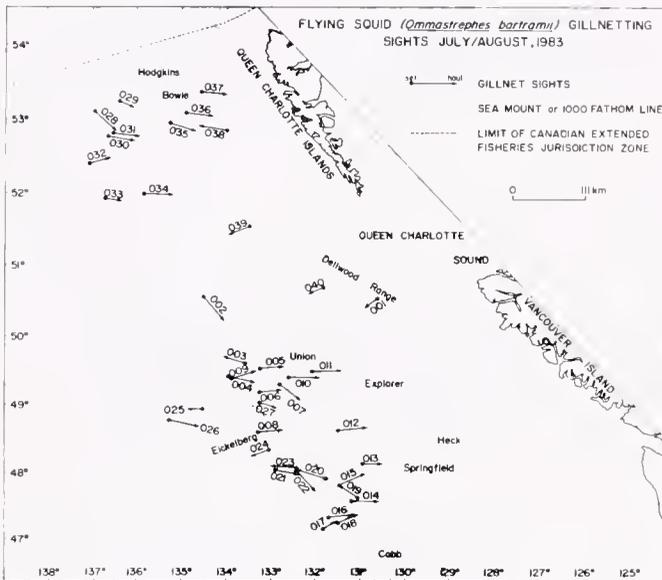


Figure 1. Map of the 40 gillnet sets between July 19 and August 31, 1983 off British Columbia. The distances between set and haul positions are to scale. Locations of the first seven nights only of jigging (July 16 to July 21) are given, thereafter jigging only occurred at gillnet sites.

Sonar was not used by the Fishing Master as he felt that 'weak' acoustic target strength and non-schooling tendency of flying squid decreased the effectiveness of detection by echo-sounding. *Ommastrephid* squids do, however, produce echo-traces (Kawaguchi and Nazumi 1972) and echo-sounding for squid is considered reliable and widely used in the northwest Pacific by the Japanese jigging fleet (Hamabe et al. 1982).

## RESULTS

### Drift-gillnet Catch

The 1376 km of drift-gillnet deployed yielded ~590.2 t of catch, of which ~56.0% was *O. bartrami* (Table 1). Squid dominated the catch of every set. The 19 species of fish by-catch was dominated by pomfret, *Brama japonica* (~188.3 t) and two shark species (~62.0 t). The 1329 salmonids taken comprised ~0.59% of the weight of total catch; among which 708 (53.2%) were Pink salmon (*Oncorhynchus gorbuscha*), 319 (24.0%) were Sockeye

TABLE 1.

Contribution of the major species or groups to the drift-gillnet catch taken off the British Columbia coast in July and August, 1983.

Species or Group	% of Total Catch*
Flying squid ( <i>Ommastrephes bartrami</i> )	55.72
Pomfret ( <i>Brama japonica</i> )	31.93
Salmon shark ( <i>Lamna ditropis</i> )	5.56
Blue shark ( <i>Prionace glauca</i> )	4.94
Albacore tuna ( <i>Thunnus alalunga</i> )	0.95
Salmonids (6 species)	0.59
Jack mackerel ( <i>Trachurus symmetricus</i> )	0.18
Others (8 species)	0.13

\* 590,175 kg.

(*O. nerka*) and 198 (14.9%) were Steelhead (*O. mykiss*). Steelhead were the most ubiquitous salmonid, occurring in 33 of the 40 sets. Approximately 65% (860) of all salmonids (66% of all *O. gorbuscha* and 95% of all *O. nerka*) were taken in the first two sets (001 and 002). The salmonid by-catch decreased strongly thereafter. Later in the season, set 040, which was about half-way between the locations of sets 001 and 002, yielded 3 salmonids.

Daily CPUE of kg squid per km gillnet ranged between 23.3 to 659.2. The CPUE of each set locality is illustrated with sea surface isotherms for that period in Figure 2. Fishing occurred in a temperature range of 13.9 to 16.3°C (median daily range of 14.6 to 16.1°C). The scale of Canadian Weather Service isotherms is too coarse to detect temperature-related patterns in CPUE. Within one set, however, the yields of individual net groups could differ according to temperature. The 6.25 km net group yields listed in Table 2 for set 014 (Fig. 2A) show that they were deployed across an unusually marked temperature discontinuity in which the squid apparently concentrated in the cooler side of the front.

To test whether there was a cycle in the CPUE (Fig. 3), the sample autocorrelation function at lag 'k' was calculated for the first 20 lags (Box and Jenkins 1976). Only the sample autocorrelation for lag (day) 14 (0.428) was significant at the 5% level. Peaks in CPUE occurred 1 to 3 days prior to full and new phases of the moon. A sharp drop in CPUE occurred at new and full moons, then a short-lived CPUE increase followed by low CPUE values during first and last quarters of the moon. Cloud cover was not recorded. There was no cross-correlation between CPUE and median daily sea surface temperature.

*Ommastrephes bartrami* ranged from 345 to 505 mm in dorsal mantle length (DML) with an approximate weight range of 1.3 to 4.3 kg. Table 3 summarized the DML data taken from 1020 individuals. The Northern sets, sampled later in the season, from August 18 to 27, yielded squid which were significantly larger (Student's t-test;  $p < 0.05$ ) than the Southern sets.

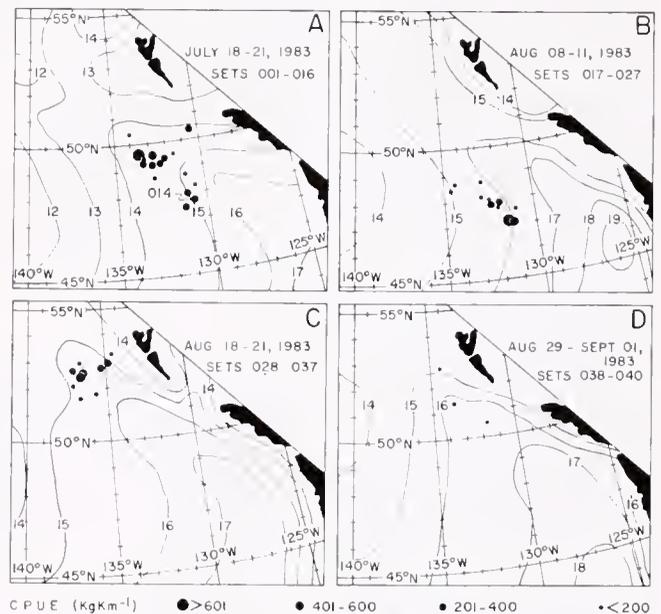


Figure 2. Canadian Weather Service sea surface isotherms (°C) off British Columbia and C.P.U.E. of *Ommastrephes bartrami* (kg squid per km drift-gillnet) at fishing sites in July and August, 1983. Dates of isotherm reports and relevant set numbers for that period are provided. The locality of set 014 is indicated in part A.

TABLE 2.

Individual yield and sea surface temperature of the seven 6.25 km drift-gillnet groups of set 014 (August 3, 1983) which yielded 11107 kg of *Ommastrephes bartrami*. For locality of set 014 see part A of Figure 2.

Order in Which Net Groups Were Deployed	% of Squid Catch in Each Net Group	Mean (range) Sea Surface Temperature of Each Net Group (°C)
1	7.7	16.0 (16.0–16.0)
2	6.7	16.0 (16.0–16.0)
3	6.1	16.0 (16.2–15.9)
4	8.4	15.7 (15.8–15.5)
5	17.3	15.6 (15.7–15.5)
6	23.2	15.4 (15.4–15.4)
7	30.6	15.3 (15.2–15.3)

#### Jigging Catch

The 820 machine-hours (41 nights) and 510 man-hours (6 nights) of jigging yielded 1.5 t of *O. bartrami*. Many of these large squid fell off the lures by autotomy or ripping of the prey-grasping tentacles in the ~3.5 m lift from sea surface to the jigging machine racks. On four nights a total of ~18 kg of the nail squid, *Onychoteuthis borealijaponica* Okada, were taken, especially in the continental shelf and slope waters of jigging sites 2, 3, and 4 (Fig. 1).

#### DISCUSSION

'Flying' squid, *Ommastrephes bartrami*, dominated the catch from warm offshore British Columbia surface waters to the most northerly extent of the survey at 53°22' latitude N during summer nights in 1983. Such high relative squid abundance has been reported from previous summers (Bernard 1980, 1981), and is probably a seasonal phenomenon. Throughout the north and northeast Pacific the summer abundance of *O. bartrami* is high as well (Araya 1983, Kubodera et al. 1983, Murata 1990). The Japanese drift gillnet fleet has expanded across the Pacific, to at least 150° longitude W, to exploit this widespread resource (Murata 1990).

The distribution of oceanic squid is considered closely related

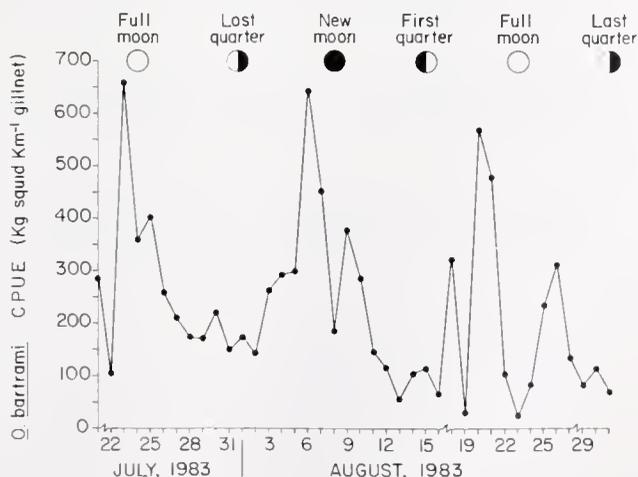


Figure 3. Catch Per Unit Effort of *Ommastrephes bartrami* according to fishing day and state of moon from July 19 to August 31, 1983.

TABLE 3.

Dorsal mantle length (DML) of *Ommastrephes bartrami* subsampled from the catch. North and South groups arbitrarily divided by 51° latitude North.

Sets	n	DML (mm)
		$\bar{x} \pm S.D.$
All sets (001–039)	1020	416.1 $\pm$ 30.5
North sets (028–037*)	270	430.1 $\pm$ 27.2
South sets (001–027)	720	410.5 $\pm$ 30.1

\* 039 excluded as midway between North and South set groups.

to water temperature (Brandt 1983, Murata 1990). Throughout the north Pacific *O. bartrami* concentrate according to the 'thermo-haline structure' of surface waters, usually  $>14^{\circ}\text{C}$ , near the Subarctic Boundary in late summer (Kubodera et al. 1983). On a large scale, therefore, *O. bartrami* are distributed according to vertical thermal gradients.

Oceanic squid are characteristically patchy in their distribution (Wormuth and Roper 1983) and on a small scale this is demonstrated in *O. bartrami* as being due to local thermal discontinuities (Table 2). We sampled only between 13.9 and 16.3°C, which is well within the preference range of *O. bartrami* (Araya 1983). The northerly distribution limits of flying squid in the northeast Pacific remain unknown. I believe *O. bartrami* could have penetrated considerably north of 53°22' N into offshore Alaskan waters during the unusually warm sea surface conditions in the summer of 1983.

In the northwest Pacific, the *O. bartrami* fishing season begins in July, peaks between August and October and declines by December as stocks of mature individuals migrate southward in preparation for spawning in subtropical waters (Murata et al. 1983, Murata 1990), such as Hawaii (Young and Hirota 1990). A similar season would probably apply for *O. bartrami* in the northeast Pacific off British Columbia. There is insufficient data, however, to assess full duration and peak abundance times for *O. bartrami* in British Columbia waters.

The interannual sea surface temperature fluctuations off the British Columbia coast probably influence the squid stocks as they do for salmonids (Tabata 1984) and albacore tuna *Thunnus alalunga* (Ketchen 1980). A longer time series of data are required in the northeast Pacific, but in the northwest Pacific sea surface temperature-related interannual variation in *T. pacificus* and *O. bartrami* stocks are well known (Kitano 1979, Kubodera et al. 1983, Murata 1990).

The CPUE of *O. bartrami* improved significantly at 14 day intervals, just prior to new and full phases of the moon. The three peaks in CPUE varied from 1 to 3 days before new and full moons, however a longer time series of observations will be needed to demonstrate lunar-related increases in surface-water abundance of *O. bartrami*. Bernard (1981) suggested that *O. bartrami* occur at depth (to 400 m) during the day. Upward migration may occur at dusk, although this is speculative as reports on the vertical migratory patterns of ommastrephid squids are limited (Roper and Young 1975). The marked decreases in abundance of *O. bartrami* at the full moons are similar to those of other oceanic squid species whose catch rates drop sharply during times of full moon (Baker 1960, Smith 1983). The decreased occurrence of *O. bartrami* during the new moon is, however, not well documented among other oceanic species.

The large dorsal mantle lengths of *O. bartrami* taken in northern sets compared to those taken in southern sets may indicate an older mean age, by weeks, of the northerly squid. *O. bartrami* grow extremely rapidly on their northward summer migration (Naito et al. 1977, Murakami et al. 1981, Araya 1983, Murata 1990) and latitudinal changes in size composition occur in the northwest Pacific (Kubodera et al. 1983).

Although Kubodera and Yoshida (1981) reported that gill nets between 42 to 157 mm stretch mesh are more efficient for *O. bartrami* than salmonids, certainly the 118 to 121 mm mesh used here readily caught salmon. The warm temperatures (14.0 to 14.7°C) did not preclude the pink and sockeye salmon by-catch despite the latter's alleged preference for waters <12°C (Tabata 1984). The high salmonid by-catch in the first two days of gill-netting could relate to relatively close proximity to shore (set 001), or too far north (set 002), so early in the summer (mid-July). As the summer progressed, salmonid by-catch decreased as fish entered inshore waters, likely in preparation for migrating up rivers. Sets more offshore and southerly in the early summer, would have better avoided incoming salmonids from the central north Pacific. Sets occurring progressively more northerly, and perhaps more inshore, through August to October would likely decrease salmonid interception.

By-catch of albacore, *T. alalunga*, was low despite the warm water fishing. Canadian waters may be the northern limit of the species (Ketchen 1980). Approximately 32% (or 188 t) of the total catch was pomfret, *B. japonica*, which was discarded because of its low market value compared to squid. This ubiquitous and abundant species occurs throughout the north Pacific (Shimazaki and Nakamura 1981) and is edible.

The nail squid, *O. borealijaponica*, was not taken in gillnets, yet Kubodera et al. (1983) report this species as second in abundance to *O. bartrami* in the north Pacific. Nail squid are generally

found in colder water (<12°C) than *O. bartrami* and when the two species co-occur they are often vertically stratified with *O. borealijaponica* being deeper (Kubodera et al. 1983, Okutani and Murata 1983). Nail squid are smaller, usually <360 mm in dorsal mantle length, and are more readily caught in the 48- to 157-mm mesh range used by Kubodera et al. (1983); of which 70% was <118 mm DML. Moreover, Bernard (1980) suggested that in British Columbia waters nail squid are found more inshore than flying squid and are more associated with continental shelf areas.

Although there is a well established northwestern Pacific jigging fishery for *O. bartrami* with significant landings (Murata 1990), jigging was ineffective during this survey. Escapement from lures during inhauling was probably a minor contributing factor. The main reasons for low jigging catches remain unknown.

In summary, *O. bartrami* occurs in offshore British Columbia waters which may relate to annual mass warm water movements. There is, however, little biological data on northeast Pacific populations and a better understanding of interannual variability in abundance, growth, migration, and interrelations with fin fish stocks is needed.

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## THE DECLINE OF THE VIRGINIA OYSTER FISHERY IN CHESAPEAKE BAY: CONSIDERATIONS FOR INTRODUCTION OF A NON-ENDEMIC SPECIES, *CRASSOSTREA* *GIGAS* (THUNBERG, 1793)<sup>1</sup>

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**ABSTRACT** The Chesapeake Bay oyster fishery for *Crassostrea virginica* (Gmelin) is in a state of continuing decline. Two diseases, *Haplosporidium nelsoni* and *Perkinsus marinus* have effectively eliminated oysters from many sections of the Bay. Despite over 30 years of disease activity the native oysters have developed neither tolerance nor absolute resistance to these diseases, and do not exhibit any recovery in disease endemic areas in Virginia. Repletion programs have completely failed to recover to permanent production areas lost to disease. Present fishery management activities are limited to a controlled retreat away from the disease in an arena where disease distribution is salinity and temperature (and hence climate) related and, therefore, beyond human influence. Disease resistance is the pivotal issue. This commentary builds on the reality that without resistance to both diseases no recovery to sustained, stable production on all formerly productive oyster bottom is possible. It is improbable that such resistance can be developed in *Crassostrea virginica*. A consideration is made of the case for introduction of a non-endemic species, *Crassostrea gigas* (Thunberg) to assist in attaining this goal.

**KEY WORDS:** *Crassostrea gigas*, oyster, introductions

### INTRODUCTION

The premeditated movement of aquatic species for aquaculture and fishery enhancement purposes has been an active component of animal husbandry for over two thousand years. Present day activity is essentially international in scope. Stimuli for such movements are many and variable, from biological control to development of local and national economies to revitalization of depressed economies suffering from native species depletion caused by disease, overexploitation, pollution or some combination thereof. Elton (1958), in his classic text on introduced species, comments on the extensive movement of oysters around the globe as part of commercial fishery activity. In this commentary we examine arguments for introduction of the Pacific or Japanese oyster, *Crassostrea gigas* (Thunberg), to Chesapeake Bay to supplement production that is currently supported only by depleted stocks of native *Crassostrea virginica* (Gmelin).

Comprehensive guidelines for consideration of and effecting introductions have been developed independently by ICES (International Council for the Exploration of the Seas), EIFAC (European Inland Fisheries Advisory Commission) and AFS (the American Fisheries Society). These guidelines emphasize the following:

- (a) a clear rationale for introduction,
- (b) selection of candidate species, including a consideration of associated pests, parasites and diseases,
- (c) testing, utilizing quarantine systems, before a decision to proceed with introduction,
- (d) introduction using quarantine procedures and monitoring after release to provide data for subsequent considerations for introductions.

Our commentary will focus on items (a) through (c) of the above list, including a brief discussion of the legal climate in this particular case, and conclude with a description of future efforts in

data collection to allow a balanced decision concerning large scale fishery rejuvenation efforts in Virginia.

### *Developing the Rationale: Historical Perspective and Current Situation*

Why should an attempt be made to restore or rejuvenate the oyster resource of Chesapeake Bay? Although the initial, and perfectly defensible, response to this question would probably be because it supports a commercially valuable industry we believe that the direct commercial exploitation aspect is of quite secondary importance. Benthic communities of Chesapeake Bay in precolonial times were dominated by intertidal oyster reefs. Oyster reefs were important geological as well as biological structures. Reefs supported extensive communities that, in turn, provided the base levels of food webs that eventually support commercially important finfish and crab species, important trophic interactions that are often underestimated in current attempts to "manage" finfish and crab stocks on a species by species basis. Demise of this productive benthic community has perhaps resulted in comparable demise of the commercial finfish and crab stocks. Limiting fishing effort on other species will have only marginal positive impacts. Further, the role of the oyster in harvesting primary productivity in Chesapeake Bay cannot be understated. The calculations offered by Newell (1989) are illuminating—a two order of magnitude decrease in filtration capacity compared to pre-1870 oyster stocks! Whereas the resident oyster population once had the capacity to filter the waters of the bay in 3.3 days, the present stocks can only manage the same task in approximately 325 days—and the stocks are still declining. A healthy and substantial oyster stock in Chesapeake Bay would probably be the single most effective mechanism of simultaneously harvesting microplankton, reducing the impact of eutrophication, sustaining a directly harvestable resource, improving water quality and maintaining a diverse and stable food web. Unfortunately, four centuries of neglect, mismanagement and wholesale mining of the oyster resource (both living and shell, the latter for industrial purposes—see Haven, Hargis and Kendall 1978, Kennedy and Breisch 1981) has resulted

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in the present scenario where sparse, disease ravaged populations survive in disparate, low salinity sanctuaries as subtidal crusts of living material overlaying a base of reef material. The importance of the oyster as a cornerstone species in Chesapeake Bay surpasses that of the directed fishery in both ecological and economic terms, yet it is the latter that embodies a disproportionate political power and which, by default, will eventually drive decision processes concerning restoration and rejuvenation including possible introductions. With this political reality clearly stated we will proceed with a greater focus on the directed commercial fishery aspect of the discussion.

The oyster (*Crassostrea virginica*) resource of Chesapeake Bay has been in continuing decline since the turn of the century (Haven, Hargis and Kendall 1978, Kennedy and Breisch 1981, Hargis and Haven 1988). Prior to 1960, average annual oyster production was 3.5 million bushels in Virginia and 2.2 million bushels in Maryland. Virginia oyster production in the 1980s decreased from over 1.0 million bushels in 1981 to 209,000 bushels in 1989. Current estimates for public fishery market oyster production in Virginia in the 1990–91 season are at an all time low of 43,000 bushels. The continuing decline due to overfishing has been assisted by the action of two diseases, *Haplosporidium nelsoni* (commonly known as MSX) and *Perkinsus marinus* (commonly known as "Dermo"). *Haplosporidium nelsoni* and *P. marinus* were at record high levels of abundance during 1986 and 1987 as a result of continuing drought conditions over the Chesapeake Bay watershed (Burreson and Andrews 1988). During 1986 and 1987, estimated overall mortality on public beds in Virginia was between 70 and 90% each year, the highest values recorded in 28 years of continuous monitoring (E. M. Burreson, unpublished data). During 1988 *P. marinus* spread to all monitored oyster beds in the Virginia portion of Chesapeake Bay. Since that time some abatement has occurred in low salinity areas (Burreson, unpublished data, May 1991) but the disease remains endemic to the majority of formerly productive oyster bottom. The combined effect of both oyster diseases has been the recent elimination of commercial oyster production from essentially all waters in the Virginia portion of the bay with the exception of three oyster bars in the upper James River and very limited areas in the upper Rappahannock River. Many oyster bars in the Maryland portion of the bay have also been denuded by the diseases. The remaining locations in Virginia, about 5% of the total public oyster grounds, are the subject of continuing, intense fishing pressure. Between 1987 and 1989 approximately 90% of the entire Virginia harvest came from the upper James River, although this declined to approximately 68% in the 1990–91 public oyster season. The magnitude of destruction and the economic implications are obvious.

In order to allow recolonization of formerly productive oyster beds, the distribution of diseases must be forced in a downstream direction by a decline in ambient salinity due to increased streamflow in the tributaries of Chesapeake Bay. Conditions typical of the 1950–1980 period still result in large, salinity related disease endemic areas and associated unproductive oyster bottom. Given the drought conditions of the 1980s in the middle Atlantic region, which exacerbated disease related losses, a marked and sustained change to wetter climatic conditions in the watershed is needed. Current, admittedly limited, understanding of the impacts of predicted global warming suggest this is unlikely. Furthermore, even a temporary increase in rainfall would result in only a temporary reduction in disease pressure. The life cycle and growth of the native oyster are such that even colonization of a presently de-

nuded, high salinity oyster bed would require a minimum of three years without serious disease losses before a single crop of marketable oysters would be attained. Clearly, management around typical, rather than atypical, rainfall and streamflow conditions is unpredictable and imprudent.

The subject of natural disease resistance and the development of disease resistance in cultured stocks of the native oyster, *Crassostrea virginica*, has received considerable attention. Distinction should be made between tolerance to a greater parasite burden, wherein mortalities will eventually occur but at a decreased rate, and resistance, where no parasite related losses are observed. The notion that disease resistance would allow recolonization of presently barren areas, with the ensuing rejuvenation of the industry, is untenable with respect to Chesapeake Bay for several reasons. Natural populations, with their enormous fecundities, have failed to produce extensive beds of tolerant, let alone resistant oysters through natural selection as demonstrated by the continued and almost total absence of oysters from high salinity areas of the bay. This is probably due, at least in part, to the large gene pool of unselected oysters, especially for *H. nelsoni*, in the upper reaches of the major tributaries in Virginia and in the upper portion of the bay in Maryland. Efforts at Rutgers University to select such strains by manipulative breeding have resulted in some improvement in survival in response to challenge by *H. nelsoni* after 25 years of research and over eight generations of selection (Ford and Haskin 1987). Improvement in survival in response to *H. nelsoni* challenge is not correlated with the activity of a particular cellular or humoral defense mechanism (Douglass 1977, Ford 1986), but appears to be the result of an overall physiological superiority in which tolerant oysters, by more efficiently utilizing available energy, are able to inhibit the development of the disease (Myhre 1973, Newell 1985, Barber, Ford and Haskin 1988a,b); however, these strains are potentially useless in Chesapeake Bay because of the presence of *P. marinus* as well as *H. nelsoni*. Resistance to both diseases, as opposed to tolerance of a higher parasite number, is essential to reestablishing stable oyster populations on all formerly productive oyster bottom in the Virginia portion of Chesapeake Bay. The unusual intensification of both diseases in recent years and the resulting high oyster mortality dictate that the time required to select native *C. virginica* for disease tolerance and, eventually, resistance using traditional methods may not be adequate to deal with current economic needs. Alternative approaches to restore a productive resource and thereby rejuvenate the industry must be considered. The introduction of a non-endemic oyster species to reestablish productive bottom in currently denuded, disease endemic areas, is such an alternative.

#### *Legal and Permitting Requirements Related to Introductions of Non-endemic Species: Can Introductions Be Effected in Virginia?*

Federal and state legislation applies in two related areas. These are respectively: experimentation with non-endemic species, compliance with ICES guidelines and U.S. Federal Law (the Lacey Act); and permitting requirements for study of non-endemic species in the Commonwealth of Virginia. U.S. Federal Law, in the form of the Lacey Act Amendments of 1981, Public Law 97-79, contains provisions for control of movement of non-endemic species into the U.S.A. and across state lines. In essence the Lacey Act is complied with if approval for possession is obtained at the state level. The appropriate section of the "Laws of Virginia relating to the Marine Resources of the Commonwealth: 1984 Edi-

tion" are found under section 28.1-183.2 entitled "Importing Fish or Shellfish for Introduction into Waters of the State." Such importations are unlawful unless written permission is obtained from the Commissioner of the Virginia Marine Resources Commission—the designated state regulatory agency. A written request containing all pertinent information (i.e., species, origin, quantities, time period, etc.) must be submitted at least 30 days prior to importation. The Director of the Virginia Institute of Marine Science must approve all requests prior to approval by the Commissioner. Provided appropriate permission is granted by the aforementioned Director and Commissioner then the legal prerequisites are fulfilled.

Neither the Lacey Act nor the Laws of Virginia address the legal and moral obligations of either informing or even seeking comment on proposed introductions from neighbouring legal jurisdictions if they are likely to be affected by such introductions. Indeed, there appears to be no specific instructions requiring such action. Formal interstate advisory and management bodies do exist but their legal authorities on the issue of introductions appear limited. Although the present discussion focusses on the Virginia portion of the Chesapeake Bay, any introduction of reproductively active, non-endemic species will potentially have impact in both Maryland and North Carolina waters if pelagic larval stages are widely dispersed and survive. Even wider geographical impact may occur over time in the event of establishment in the recipient environment. Clearly, the ability of neighbouring states to influence the permitting process through alternate legal challenges remains untested.

#### *Selection of Species for Introduction: Why Crassostrea gigas?*

When considering the selection of species for introduction it is important to effectively match the donor and recipient environments to insure greatest possibility of successful survival of the introduced species. The Chesapeake Bay environment can be characterized as having a continental climate with large air and water temperature ranges; large temporal and spatial salinity variation; a geologically young, sedimentary basin that has been extensively dredged to facilitate past and current commercial shipping; a region where salinity related endemic diseases currently limit native oyster distribution, and an irretrievably altered watershed that currently serves as home to over 14 million people. In summary, this is a high stress environment that is drastically altered from that prior to colonial settlement—the environment in which *Crassostrea virginica* flourished to form reefs that were major geological features as well as dominant components of the benthic community of Chesapeake Bay. The magnitude of change over the past four centuries should be underscored. Despite continuing efforts to improve water quality in the bay it must be realized that the cumulative abuses of urban and agricultural development to the bay watershed make the goal of restoration of the bay to its former pristine condition (as described in Captain John Smith's logs) untenable. Intertidal oyster reefs no longer exist in the bay, they have been tonged and dredged to subtidal depths generally exceeding one meter. The quantitative change in oyster reef structure associated with their degradation from intertidal to subtidal features is illustrated by the fact that present, immediate subsurface shell deposits have been radiocarbon dated at several hundred years before present (DeAlteris 1988).

It is appropriate to begin a search for an alternate species within the genus *Crassostrea*—reef forming species tolerant of mid to

subtropical latitude, high stress environments. Tables 1–3 summarize species in the genus *Crassostrea*, and compare published data describing their temperature and salinity tolerances as both larval and adult forms. Caution must be applied in literature review in determining the geographic origin of *C. virginica* under examination (see comments in Hedgecock and Okazaki 1984, Reeb and Avise 1990, concerning lack of genetic uniformity throughout the zoogeographic range of this species), and, where possible, which geographic type of *C. gigas* (there are four, named by prefecture of origin, Hokkaido, Myagi, Hiroshima and Kumamoto, see comments in Torigoe 1981, Quayle 1989, Kusuki 1990) is being described. Geographic types of *C. gigas* are characterised by distinct growth rates and forms (so much so that they serve quite different commercial markets) that may have different temperature and salinity optima and tolerances. Such information on geographic type is rarely given, therefore data in tables 1–3 encompasses all types. For the present comparative purpose this is acceptable in that it may overestimate rather than underestimate possible ranges of *C. gigas* in the Chesapeake Bay. In general, the Myagi strain has been the focus of work in the hatchery based fishery of the Pacific coast of North America; however, there has been much intentional interbreeding of introduced stocks and precise pedigrees are lacking. The predominant oyster of that and the European fisheries can better be described as Myagi-like. Several other species lack adequate documentation for complete comparison; however, it is evident that strong similarities exist between *C. virginica* and *C. gigas*.

*Crassostrea gigas* is actively cultured elsewhere in the world, especially so as an introduced species. *Crassostrea gigas* has been extensively (both accidentally and intentionally) moved beyond its native oriental range for culture purposes to locations in the Pacific basin (Costa Rica through Alaska, Australia, New Zealand), and the Atlantic basin (North Sea through Mediterranean and Atlantic Coast of Morocco). Comprehensive summaries of these activities are given in Mann (1979, 1981) and Menzel (1990). *Crassostrea gigas* is the basis of the largest oyster fisheries in the world. During 1987 the leading oyster producing countries in the world were Korea and Japan with production of 303,233 and 258,776 metric tons respectively, this product being predominantly

TABLE 1.

**Crassostrea species: Distribution and Synonyms. Source material: 1. Ahmed, 1971; 2. Boffi, 1979; 3. Carreon, 1969; 4. Chen, 1972; 5. Dang, 1972; 6. Durve, 1967; 7. Kamara et al., 1976; 8. Kong and Luh, 1977; 9. Mann, 1981; 10. Menzel, 1974; 11. Newball and Carriker, 1983; 12. Shafee and Sabatie, 1986; 13. Tebble, 1966; 14. Torigoe, 1981; 15. Zenkevitch, 1963.**

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Atlantic coast of North America: <i>virginica</i> (= <i>rhizophorae</i> ), 11.
Brasil: <i>brasiliensis</i> (= <i>rhizophorae</i> = <i>virginica</i> ?), 2, 7
Western Europe, English Channel to Morocco (now rare): <i>angulata</i> , 10, 13.
Europe, North Sea through Mediteranean to Morocco: <i>gigas</i> , 9, 12.
Pacific coast of North America: <i>gigas</i> , 9, 12.
Japan, Korean Peninsula through Vietnam: <i>gigas</i> , <i>araiensis</i> (= <i>rivularis</i> ), <i>nippona</i> , 5, 14.
India: <i>gryphoides</i> , <i>madrasensis</i> , <i>rivularis</i> (= <i>araiensis</i> ), 1, 6.
Thailand/Malaysia: <i>belcheri</i> (= <i>nippona</i> ?), 4, 8.
Philippines: <i>iredali</i> (= <i>madrasensis</i> or even = <i>rivularis</i> ?), 3.
West Africa: <i>gasar</i> (= <i>tulipa</i> ), 7.
Black Sea: <i>taurica</i> , 15.

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TABLE 2.

Temperature and salinity ranges of adults of *Crassostrea* species. Optimum ranges given in parentheses.

Species	Temperature (C)		Salinity (ppt)		Reference
	Growth	Spawning	Growth	Spawning	
virginica	5–34 (28–32)	18–25 (23)	>5 (12–27)	>8	7,8,20,21,22,31
angulata	20–30	20	21–43	<33	3,4,16
araiakensis		7–40 (30–40)			5,11,16
gasar	25–30	5–34	14–20		1,28,29
gigas	3–35 (11–34)	16–30 (20–25)	10–42 (35)	10–30 (20–30)	2,4,15,18,19,24,25
gryphoides	19–33	27–31	4–40 (30–40)	13–29	11,13,23
iredali	30–33	<45	>15		4
madransensis	26 (30)	1–41 (8–25)	17–35 (20–35)		16,17,26,27,30
nippona	no data				
rhizophorae			22–40 (26–37)		4,5,12
taurica	3–28	17–18			32

Reference: 1. Ajana, 1980; 2. Allen et al., 1988; 3. Amemiya, 1926; 4. Bardach et al., 1972; 5. Boveda and Rodriguez, 1967; 6. Breese and Malouf, 1977; 7. Butler, 1949; 8. Chanley, 1958; 9. Davis, 1958; 10. Davis and Calabrese, 1964; 11. Desai et al., 1982; 12. Dos Santos and Nascimento, 1985; 13. Durve, 1965; 14. His et al., 1989; 15. Hughes-Games, 1977; 16. Jhingran and Gopalakrishnan, 1974; 17. Joseph and Madhyastha, 1984; 18. King, 1977; 19. Le Gall and Raillard, 1988; 20. Loosanoff, 1958; 21. Loosanoff, 1969; 22. Loosanoff and Davis, 1952; 23. Mane, 1978; 24. Muranaka and Lannan, 1984; 25. Nell and Holliday, 1988; 26. Rao, 1951; 27. Rao and Naylor, 1956; 28. Sandison, 1966; 29. Sandison and Hill, 1966; 30. Stephen, 1980; 31. Wells, 1961; 32. Zenkevitch, 1963

*C. gigas*. By comparison the United States, ranking third, produced 217,632 metric tons (a mix of *C. gigas* and *C. virginica*) and France, producing predominantly *C. gigas* after initial introduction of the species some 15 years earlier, ranked fourth at 123,162 metric tons. *Crassostrea gigas* is elegantly suited for hatchery production as demonstrated by the enormous success of the hatchery-based industry in the U.S. Pacific Northwest. Commercial production based on hatchery produced seed oysters in the Northwest far exceeds present oyster production from the entire Chesapeake Bay. Domestic oyster production cannot satisfy the market need and the United States has, since 1985, held the dubious distinction of being the world's leading importer of oysters in fresh and frozen form.

The native northern European oysters *Ostrea edulis* and *Crassostrea angulata* were decimated by disease in the mid 1970s. Production of the former fell from 15,000 tons to the present day level of 2,500 tons per year. Production of the latter fell from 60,000 tons per year to zero. The industry was saved from economic extinction by the introduction of *C. gigas*. European *C. gigas* production (including French) now employs over 20,000 people and produces approximately 140,000 tons of oysters per year, this representing over 80% of the total production. Further,

*C. gigas* appears resistant to challenge by both *Bonamia ostreae* and *Marteilia refringens*, diseases that continue to decimate native European oysters. The analogies with Chesapeake Bay are painfully obvious.

#### Risk Analysis for Introduction of Diseases with *Crassostrea gigas*

The argument in support of possible use of *Crassostrea gigas* in restoration of the presently unproductive areas of the bay has, to this juncture, appeared positive. Questions of diseases associated with *C. gigas* in its native and introduced range remain—are there such diseases and could they be transferred to the bay with an introduction? *Crassostrea gigas* has, in its native range, no known diseases that have been associated with large-scale mortalities (Koganezawa 1975). In addition, it has been used successfully as an introduced species in areas where the native oysters have been decimated by diseases. *Crassostrea gigas* has been resistant to the local diseases and no new disease introductions have been positively documented even though, in certain areas, *C. gigas* has been introduced with few, if any, control measures. For example, *C. gigas* is not susceptible to *Bonamia ostreae* and *Marteilia refringens*, diseases that have caused massive mortalities in *Ostrea edulis*, the native species in western Europe, and it has not been susceptible to similar protozoan diseases where it has been introduced in Australia and New Zealand. In addition, *C. gigas* is resistant to the viral diseases that caused mass mortalities of the Portuguese oyster in France. The Japanese oyster is the basis for the hatchery-based industry in the Pacific Northwest and no new diseases (that cause measurable mortality) have been introduced into that region (Glude 1975) even though there have been periodic importations of *C. gigas* since 1902 and early introductions were effected without any control measures being enforced. Andrews (1980) reviewed oyster introductions around the world and discussed potential problems with such importations and precautions necessary to avoid disease introductions.

The extensive movement of *C. gigas* has provided, in addition to the native range, many potential sources for broodstock for a

TABLE 3.

Temperature and salinity ranges of *Crassostrea* larvae. Optimum ranges given in parentheses. Reference material as in Table 2

Species	Temperature (C)	Salinity (ppt)	Reference
virginica	20–33	8–39 (10–29)	3,9,10
angulata		21–43 (28–35)	3,4,16
araiakensis	20–28 (26–28)	10–30 (20)	5
gigas	18–35 (30)	19–35	2,14,15
rhizophorae	<30 (25)	20–40 (28)	12

no data available for gasar, gryphoides, iredali, madrasensis, nippona and taurica.

proposed introduction. For the present discussion we will essentially limit our consideration of source broodstock to that from the state of Washington. Despite the fact that the pedigrees of these stocks are not definitively documented, the stocks are mostly of Myagi Prefecture origin but many years of hatchery breeding may have resulted in some limited crossing with stocks from other sources, they do have a known and documented history concerning associated pests, parasites, and diseases. The listing below includes only those organisms reported from *C. gigas* that are actual or potential disease agents in oysters or other bivalve molluscs. It does not include the numerous parasites, mostly metazoan, found in oysters world-wide that have never been implicated in host mortality.

### 1. Diseases of Unknown Etiology.

**Hematopoietic Neoplasia.** This disease results in a massive tissue invasion of abnormal blood cells and is analogous to leukemia in vertebrates. It has been implicated in large-scale mortalities of mussels in the state of Washington and of soft-shell clams in Chesapeake Bay. The syndrome has been reported in *C. gigas*, *C. virginica*, and *O. lurida*, but has not been associated with mortality in these species. A virus has been suggested as the cause for this disease, but the evidence is weak.

**Potential implications:** This syndrome is already present in Chesapeake Bay and has been observed occasionally in *C. virginica*.

### 2. Viral Diseases.

**a. Oyster Velar Virus.** This disease affects oyster larvae and has been reported from two hatcheries in the state of Washington (Elston and Wilkinson 1985). It has been observed occasionally in hatcheries from March to August in larvae greater than 150  $\mu\text{m}$  in shell height. Infection results in loss of motility and death of larvae. Measured losses of hatchery production up to 50% have been recorded, but there is no established link between the disease and mortality since it has not been experimentally transmitted. There have been no reported outbreaks of the disease in recent years (R. A. Elston, Battelle Center for Marine Disease Control, Sequim, WA, personal communication).

**Potential implications:** This virus is primarily a hatchery problem where larvae are held at high density in tanks, but even in hatcheries the virus has never caused mortality over 50%. It is not expected to be a problem in nature where density of larvae is much lower than in hatcheries and transmission of viral particles between larvae is greatly reduced.

**b. Hemocytic Infection Virus (HIV) and Gill Necrosis Virus (GNV).** These iridoviruses have been reported from *C. gigas* in France. Both viruses were implicated in mass mortalities of the Portuguese oyster *C. angulata* in France during the 1970s (Comps and Bonami 1977), but neither virus causes mortality in *C. gigas* in the same area (Comps 1988). In fact, Comps (1988) states that the ability of *C. gigas* to resist mortality from these viruses resolved a very serious economic problem associated with the total elimination of the Portuguese oyster.

There has been some speculation that *C. gigas* is a carrier for these viruses and that one or both of them was introduced into France with importations of *C. gigas* from Japan. According to Henri Grizel, IFREMER, France, (personal communication, 12 March 1990) the lesions characteristic of the viral infections were observed in *C. angulata* prior to introduction of *C. gigas*, which

suggests that the viruses were already present in France. Unfortunately, no attempt was made to isolate viruses at that time, so we will never know with certainty if the viruses were already present.

**Potential implications:** GNV and HIV have never been observed in *C. gigas* from the Pacific Northwest. In addition, the very characteristic gill lesion caused by GNV has never been observed (R. A. Elston, personal communication, 14 March 1990).

There are many reports in the literature of other viruses in oysters and other marine molluscs, including five different viruses from the eastern oyster, *C. virginica* (Johnson 1984). There is no firm evidence that any of these viruses (other than HIV and GNV) can be pathogenic to their hosts.

### 3. Bacterial Diseases.

**a. Bacillary Necrosis.** Many species of bacteria in the genus *Vibrio* are present naturally in seawater. They are not normally pathogenic, but can become so because of adverse environmental conditions, usually high temperature. These bacteria have been implicated in often complete mortality of larvae in hatcheries from various regions of the world. Juvenile oysters have also been reported to be affected in hatcheries in Maine. Affected oyster species include *C. gigas*, *C. virginica* and *Ostrea edulis* (Elston 1984, Sindermann and Lightner 1988).

**Potential implications:** *Vibrios* and other bacteria that may cause this problem are present naturally in seawater. Rigorous hatchery sanitation measures usually are sufficient to prevent mortalities. The Virginia Institute of Marine Science oyster hatchery has experienced no problem of this type.

**b. Nocardiosis.** This disease is caused by the actinomycete bacterium *Nocardia* and often results in raised green to yellow nodules on the mantle. It is apparently at least partially responsible for the historically reported phenomenon of summer mortality in adult *C. gigas* in the Pacific Northwest (see Friedman, Beattie, Elston and Hedrick 1991). Similar nodules have been observed in other oysters from other areas, including *C. virginica* (Elston, Beattie, Friedman, Hedrick and Kent 1987), but the cause of the nodules has not been determined in those cases.

**Potential implications:** This is a husbandry disease with local environmental sources of the bacterium in Washington and British Columbia which is restricted to certain embayments. It is not a disease of major concern in those areas.

**c. Rickettsiae.** Rickettsiae are obligate intracellular organisms and have been reported from digestive diverticula epithelial cells in *C. gigas*, *C. virginica*, and many other bivalve molluscs (Kinne 1983), but are not known to be responsible for mortality.

**Potential implications:** Rickettsiae have already been reported from *C. virginica* in Chesapeake Bay.

### 4. Protozoan Diseases.

**a. *Marteilia refringens*.** This parasite has been responsible for massive mortality of the native oyster *Ostrea edulis* in France. *Marteilia refringens* has also been reported in *C. gigas* in France (Cahour 1979), but prevalence and intensity were low and only early stages of development were observed. The infections were considered to be transient and no mortality has been observed in *C. gigas*.

**Potential implications:** This parasite is known only from Europe and does not develop normally in *C. gigas*. There is little chance of importing this parasite if the broodstock is limited to *C.*

*gigas* from the state of Washington, and ICES guidelines for quarantine of broodstock are followed.

**b. *Haplosporidium* spp.** A parasite that is morphologically similar to *Haplosporidium nelsoni* (MSX) has been observed in *C. gigas* in Korea (Kern 1976). Prevalence was very low, only 0.28% in 1,438 oysters examined, and no mortality has been reported. One of the four infected oysters contained spores and they were restricted to epithelium of the digestive diverticula, as they are in *H. nelsoni*. Another haplosporidan was reported in a single *C. gigas* from California (Katkansky and Warner 1970). Spores were observed throughout the connective tissue, similar to *Haplosporidium costale* (SSO) in *C. virginica*, but spore size was intermediate between *H. nelsoni* and *H. costale*. Plasmodial stages of a haplosporidan were observed in a single *C. gigas* from Washington (Pereya 1964).

**Potential implications:** There has been speculation that the two haplosporidans from Korea and California are *H. nelsoni* and *H. costale* respectively and that they were introduced to Chesapeake Bay region with unauthorized private plantings of *C. gigas* during the 1950s; however, there is no direct evidence and it remains only speculation. There is no danger of importing these, or any other, parasites with *C. gigas* if initial broodstock are kept in quarantine and only uninfected progeny from the hatchery are used in susceptibility studies or possible introductions.

**c. *Marteilioides chungmuensis*.** This parasite infects eggs of *C. gigas* in Japan and Korea (Comps, Park and Desportes 1986). It is related taxonomically to important oyster pathogens such as *Marteilia refringens* discussed above, but *M. chungmuensis* is not known to cause mortality. This parasite may be what Becker and Pauley (1968) observed in eggs of *C. gigas* in California. Less than 10% of the eggs were infected in any one female oyster and there was no evidence of oyster mortality.

**Potential implications:** Transmission studies have never been attempted with this parasite and the life cycle is unknown; however, this parasite infects eggs suggesting that quarantine of broodstock may not provide sufficient control. This parasite is apparently not pathogenic and it has never been reported from the Pacific Northwest.

**d. *Mikrocytos mackini*.** This parasite infects vesicular connective tissue cells and causes abscess-type focal inflammatory lesions in the mantle and gonad of *C. gigas*. It is known only from British Columbia, Canada although a similar parasite has been observed in *C. gigas* from Hawaii (Farley, Wolf and Elston 1988). Average mortality of 34% was observed during early occurrences of the disease before growers learned proper management techniques to avoid mortality (Bower 1988). Oysters less than two years of age are not affected and mortality of older oysters is reduced when held high in the intertidal zone.

**Potential implications:** This parasite is not known from the state of Washington. Quarantine of broodstock and use of progeny for field studies would prevent introduction of the parasite even if it were present.

## 5. Metazoan Parasites.

***Mytilicola orientalis*.** This highly modified copepod inhabits the digestive tract of *C. gigas* in Japan. It was introduced to the Pacific Northwest with early shipments of *C. gigas* seed from Japan and is now endemic along the west coast of the United States (Sindermann and Lightner 1988). This parasite has been implicated in sporadic mortalities of *C. gigas*, but the evidence has

never been very strong. A recent, thorough, ten year study (Davey 1989) on a related species in mussels found no evidence of host mortality and the author argues forcefully that *Mytilicola* has been wrongly indicted in previous mortalities.

**Potential implications:** This parasite infects adult oysters and can be easily controlled by quarantine of broodstock in the hatchery.

In summary, quarantine of broodstock in a hatchery and the use of first generation offspring for any field studies, that is compliance with ICES guidelines for introduction of non-native organisms, will prevent introduction of all disease agents listed above except viruses, bacteria and the ovarian parasite *Marteilioides chungmuensis*, which is not known to cause mortality. If broodstock were limited to one source, the state of Washington, such problems could be minimized in that no pathogenic viruses are known in adult *C. gigas* from Washington and *M. chungmuensis* is absent from that area. There are no published reports of a serious disease outbreak in *C. gigas* from Washington and there are no documented disease introductions (that have resulted in measurable mortality) from the numerous introductions of *C. gigas* that have occurred around the world. Some incidental parasites have been introduced, but such introductions would not have occurred if ICES guidelines had been followed.

## *Susceptibility of Crassostrea gigas to Diseases Endemic to Chesapeake Bay: Perkinsus marinus and Haplosporidium nelsoni*

Of the two diseases endemic to the bay *Perkinsus marinus* is the only one amenable to laboratory experimentation. *Haplosporidium nelsoni* challenge can only be adequately effected by in situ exposure in *H. nelsoni* endemic areas. All stages of *P. marinus* are infective and the addition of finely minced, infected oyster tissue has been found to be very effective at initiating new infections in previously unexposed oysters in laboratory systems (Meyers, et al. 1991).

The susceptibility of both *C. virginica*, originating from Mobjack Bay broodstock, and *C. gigas*, F1 animals cultured at Gloucester Point, VA from a broodstock imported from Washington state in February 1989 and maintained in quarantine under ICES guidelines throughout study, to *P. marinus* was examined in two separate experiments by Meyers, et al. (1991). In the first experiment of 83 days duration 40% of the *C. gigas* became infected compared to 100% of the *C. virginica*. In the second experiments prevalence was high in both species after 60 days, but differed in intensity with moderate to high levels in *C. virginica* but low levels in *C. gigas*. Cumulative mortality over a 150 day period was 100% for *C. virginica* but only 25.1% for *C. gigas*. Other evidence suggests that *C. gigas* mortalities were not disease related. In summary, *C. gigas* consistently exhibited much higher tolerance of *P. marinus* than did *C. virginica*.

Where non-endemic material is introduced to a quarantined system for subsequent disease challenge the question arises as to the status of the stock before challenge begins. The ICES procedures are designed to preclude the possibility of vertical transmission of a disease from the introduced parent stock. Experience with application of ICES guidelines with oyster movements elsewhere, through the Conwy laboratory in the United Kingdom for example, indicates their effectiveness. Given the continuing quarantine maintenance regime for *C. gigas* in our laboratory, where sanitation procedures limit water and food availability and thereby provide continuing stress on maintained animals, it is probable that

disease, if present, would have already manifested itself; however, no evidence of disease organisms has been seen in histological sections of sampled animals.

#### *The Dilemma: Where to Now?*

To this point we have presented arguments to support the following:

- (1) Native oyster populations continue to be decimated by endemic diseases, leaving large areas of formerly productive bottom unproductive in disease endemic areas.
- (2) Current management practices have failed to reclaim to permanent production areas lost to disease.
- (3) Selected strains of native oysters, developed at Rutgers University, have developed tolerance to *H. nelsoni*; however, the surviving population in the Chesapeake Bay has developed neither tolerance nor resistance to the two endemic diseases when they occur in combination as demonstrated by their absence from disease endemic areas.
- (4) It is timely to consider another oyster species that may have improved tolerance or resistance to the endemic diseases to assist in reclamation of currently unproductive bottom.
- (5) A survey of the available literature, although limited, suggests that *Crassostrea gigas* has salinity and temperature tolerances similar to the native oyster.
- (6) Laboratory challenges of *Crassostrea gigas* with *Perkinsus marinus* strongly suggest that it is much more tolerant than the native species of oyster.

From this basis we will proceed to present arguments in favor of continuing examination of the proposed introduction and the benefits that will accrue. It is important to underscore that any further pursuit of this line of investigation in terms of disease challenge will necessitate de facto introduction of *Crassostrea gigas* into Chesapeake Bay waters. This is the only way to effect meaningful challenge with *H. nelsoni*. Despite the availability of ICES protocols to insure practically minimal introduction of associated pests, parasites and diseases, and triploid induction techniques to minimize spawning (review by Beaumont and Fairbrother 1991), there is no practical manner to absolutely insure that no spawning of stock introduced for experimental purposes will occur. A comprehensive examination of such issues as temperature and salinity tolerances of the various life history stages of *C. gigas*, and laboratory examination of susceptibility to local predators and physical environment can only provide greater ability to evaluate possible establishment and range extension in Chesapeake Bay. They cannot provide an avenue to eliminate the possibility of spawning. In situ *H. nelsoni* challenge of *C. gigas* has already been the subject of pointed debate among academics, regulatory bodies and industry at both an intra and interstate basis. Effecting such a study cannot be accomplished without limited risk of development of a self sustaining, resident population of *C. gigas* in Chesapeake Bay. Proceeding with such *H. nelsoni* challenges are an integral and necessary component of identification of disease tolerant or resistant stocks, be they of native or non-endemic origin. Eventually, a balanced decision must be made by regulatory agencies concerning the competing pressures to expedite rejuvenation of an ailing industry and consider the unpredictable biological consequences of introduction of a non-endemic species.

A major source of debate subsumed in the question of in situ testing is the possible impact of a resident *C. gigas* population in

Chesapeake Bay and competitive interaction with the native species, *C. virginica*, both within the bay and potentially outside the bay if *C. gigas* were to spread to either the north or the south of the bay mouth. During the period 1940 through 1960 testing of *C. gigas* was conducted in the lagoon systems of the Delmarva peninsula and Delaware Bay. Resident populations have not resulted although these may have been precluded by the nature of the introductions. Adequate documentation is unavailable. The Delmarva coastal lagoons and intertidal flats still maintain considerable oyster resources. On the Atlantic seaboard north of the mouth of Delaware Bay, where *P. marinus* is absent, the native oyster continues to exist as disjunct populations of various sizes, but always at levels well below historical records. These regions have again suffered variously from disease, including *H. nelsoni*, sustained harvesting and degrees of environmental degradation. Recent efforts to revive the Connecticut oyster industry through extensive shell planting and resource management are meeting with some success. Limited, culture based production exists in New England, and both cultured and wild caught oysters are available from the Canadian Maritime provinces. Investigations at Rutgers University, described earlier, concerning increased tolerance to *H. nelsoni* offer some hope of expanded oyster production in this geographic region but large scale production and reintroduction of the native species remains an enormous task. With respect to possible establishment of *C. gigas* south of Chesapeake Bay, the data of tables 2 and 3 are of limited use in estimating range extension in that definitive temperature and salinity tolerance tests have not been published for *C. gigas*. Such data are clearly desirable. Some further information may be obtained from detailed examination of current oriental culture practices within the native range of *C. gigas* (see Kusuki 1990); however, caution must again be applied in determining which geographic type of *C. gigas* is being described.

Competitive interactions in a two species scenario in Chesapeake Bay with *C. gigas* in higher salinities and *C. virginica* in lower salinities are difficult to predict because only a few meaningful analogies exist. One such analogy is the Chinese culture of *C. gigas* relative to that of the Suminoe oyster, *Crassostrea rivularis*. The latter species is, like the Myagi type of *C. gigas*, fast growing and often quite large; however, it is generally acknowledged by Chinese workers (personal communication to Roger Mann) to tolerate lower salinities. What limits the distribution of each of the *Crassostrea* species in the Chinese fisheries? This is not adequately documented, thus limiting our predictive capability for Chesapeake Bay if a reproductively active population of *C. gigas* is introduced. The second analogy is the estuarine environment of the Gironde on the Charente River in western France (the major seed oyster producing area for *C. gigas*) and in south west France where harvest pressure is comparatively light, allowing greater densities of oysters to develop (Heral and Deslous-Paoli 1990). The former location can be used as an analogy to the James River seed oyster beds and the latter location as an analogy to a situation in Chesapeake Bay where *C. gigas* is introduced as a reproductively active population to currently unproductive bottom in disease endemic areas and allowed to proliferate without excessive harvest pressure. Such a situation would obviously necessitate several prerequisites including regulatory approval to effect in situ disease challenge, a demonstrated resistance to *H. nelsoni*, and a further regulatory decision to effect refurbishment by release of reproductively active *C. gigas* cultured through ICES protocols. The argument for a comprehensive examination of both the Chi-

nese and French sites is compelling. The third and final region of interest is Queensland, New South Wales and Victoria in Australia where the introduced *C. gigas* is competing with the native and highly prized Sydney rock oyster, *Saccostrea (Crassostrea) commercialis* (review by Pollard and Hutchings 1990). Unlike the French or Oriental situations, this Australian site allows a unique opportunity to study a confrontation of an introduced and native species in progress, where *C. gigas* is the introduced species of interest. In this situation we can pointedly examine the predictive value of temperature-salinity tolerances or similar physical data relative to other biological variables such as spawning and settlement periodicities. At present the further spread of *C. gigas* in New South Wales is controlled by the management activity of removing oyster settlement substrate shortly after settlement occurs (P. H. Wolf, Dept. State Fisheries, N.S.W., Australia; personal communication to Roger Mann). *Saccostrea commercialis* is more tolerant of exposure than *C. gigas* and selective mortality occurs before the substrate is returned to the water. Whether or not *C. gigas* and *S. commercialis* could eventually coexist if control activity ceased remains unanswered, although it is relevant to note that *C. gigas* is now cultured in preference to *S. commercialis* in New Zealand due to its higher growth rate and comparable market price, and a substantial fishery for *C. gigas* now exists in Tasmania (Pollard and Hutchings 1990).

There is little question that the future of the Virginia oyster industry in its present form is very bleak if a disease resistant oyster is not identified. In addition to the biological impacts, the sociological, political and economic impacts of a continuing decline in oyster production are widespread and demand responsible action in a viable time frame. Identification of a disease resistant oyster is only the beginning of the solution, irrespective of whether that be *C. gigas* or any other species of oyster. If disease resistance

is demonstrable and a decision to proceed with introduction is forthcoming, then a hatchery based program functioning under ICES protocols must be implemented on a sufficient scale to provide seed in a timely manner to maintain and rebuild the depressed resource and the industry it supports. The present industry relies upon a naturally reproducing resource and a critical decision would relate to development and protection of actively spawning broodstock regions, similar to that operated in the Gironde, rather than the clearly untenable option of attempting to continually supply seed for extensive planting in the current "put and take" mode of operation. Alternatively, utilization of triploid oysters, both native and otherwise, in species specific, intensive culture operations may be economically attractive. Rejuvenation of the Virginia oyster industry is a task of immense proportions and will require revision and diversification of many current practices if formerly unproductive bottom is to be reclaimed to stable production, and production levels increased to allow continued competitiveness in an international marketplace for the end product. Based on the available information we believe that serious consideration should be given to the utilization of an introduced species, *C. gigas*, as part of that effort.

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## TEMPORAL VARIATIONS IN SPAWNING BEHAVIOR OF SEA SCALLOPS, *PLACOPECTEN MAGELLANICUS* (GMELIN, 1791), IN THE MID-ATLANTIC RESOURCE AREA<sup>1</sup>

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**ABSTRACT** Interannual variation in spawning of *Placopecten magellanicus* (Gmelin) may be important to management agencies concerned with maximizing yield-per-recruit via restrictions on meat counts or temporal restrictions on catch and effort. In this study, temporal patterns in the spawning behavior of sea scallops in the Mid-Atlantic resource area for the period April 1987–April 1991 are analyzed using conventional time-series methods. Biannual spawning was found to be characteristic of sea scallops in the Mid-Atlantic resource area but was also found to be erratic in the timing, duration, and magnitude. The spring spawning event was the more predictable and dominant spawning event. The fall spawn was temporally-erratic; it did not occur in 1989. Longer time-series and analyses of environmental factors are recommended to more precisely determine the gametogenic and spawning cycle of sea scallops in the Mid-Atlantic resource area.

**KEY WORDS:** temporal spawning behavior, *Placopecten magellanicus*, gonadal weight

### INTRODUCTION

The Mid-Atlantic resource area has become increasingly important to the United States sea scallop, *Placopecten magellanicus* (Gmelin), commercial fishery. The Mid-Atlantic resource area accounted for approximately 40% of the total reported U.S. landings of sea scallops between 1980 and 1990 (National Marine Fisheries Service 1989). Landings from the Mid-Atlantic area, however, have varied widely; between 1980 and 1990, landings ranged from a low of 1700 mt in 1982 to 7900 mt in 1987. In more recent years, landings have exhibited less annual variation.

Reasons for the variation in production between 1980 and 1986 and the subsequent reduced variation since 1987 probably reflect patterns in recruitment. The pre-recruitment and recruitment indices for the Mid-Atlantic resource area indicate considerable variation between 1980 and 1985 and consistently increasing levels since 1986 (National Marine Fisheries Service 1990). Consistently strong recruitment is thought to be a major reason why the Mid-Atlantic resource has not dramatically declined given the high level of landings in the past 5 years.

The occurrence of recruitment is not the only important consideration in assessing the relationship between resource levels and landings. DuPaul et al. (1989) and Schmitzer et al. (1991) demonstrated a biannual spawning pattern for sea scallops in the Mid-Atlantic resource area. Depending upon weather and other environmental factors, biannual spawning could affect recruitment patterns and the timing and magnitude of recruitment. DuPaul et al. (1989) and Schmitzer et al. (1991), however, only examined the 1988 gametogenic cycle; thus, it is unknown whether biannual spawning is a consistent characteristic of sea scallops in the Mid-Atlantic resource area. In addition, it is not known if there is a dominant spawning event.

Extensive studies on the gametogenic and spawning cycle of the giant sea scallop have been conducted by other researchers (Welch 1950, Posgay and Norman 1958, Naidu 1970, MacDonald and Thompson 1986, 1988, Barber et al. 1988). Most of the stud-

ies have limited attention to the gametogenic cycle of sea scallops in areas other than the Mid-Atlantic and over a relatively short time period—1–2 years. Conclusions derived from these studies may not apply to the Mid-Atlantic area or the long-run.

Naidu (1970) and other researchers (Sastry 1966, Newell et al. 1982, Rodhouse et al. 1984, MacDonald and Thompson 1988) have suggested the gametogenic cycle and spawning pattern may vary over time in response to environmental factors. As illustrated in Schmitzer et al. (1991), variations in the gametogenic cycle and spawning events have important ramifications for management of the fishery. Given the limited knowledge available on spawning behavior of scallops in the Mid-Atlantic area and the associated implications for management, there is a need to better understand the gametogenic cycle and spawning behavior over a longer period of time than considered in previous studies.

In this paper, temporal or short and long-run patterns in the gametogenic or gonadal cycle are examined. Analysis of variance and Scheffe tests are used to characterize major and minor spawning events. Univariate time-series models of monthly mean values of wet gonad weights between April 1987 and April 1991 are estimated and used to analyze the timing, magnitude, duration, and periodicity of spawning events.

### MATERIALS AND METHODS

Since April 1987, data relating to growth and gametogenesis of scallops in the Mid-Atlantic area have been collected on a weekly basis; data collection activities and sampling procedures are described in DuPaul et al. (1989), Kirkley and DuPaul (1989), and Schmitzer et al. (1991). For this study, whole fresh or unshucked scallops were obtained from commercial fishing vessels operating from south of Long Island (40° 00' N 73° 00' W) to north of Cape Hatteras (37° 30' N 74° 30' W) (Figure 1).

A total of 449 samples comprised of 19,351 observations on wet gonadal weight measured to the nearest 0.1 g were examined for this study. Data were pooled over sex and geographical areas and subsequently over 4 shell-height groups (85–89 mm, n = 5698; 90–94 mm, n = 6145; 100–104 mm, n = 4463; 110–114 mm, n = 3045). Pooling over sex and area was necessary because of the large number of observations and the inability to visually

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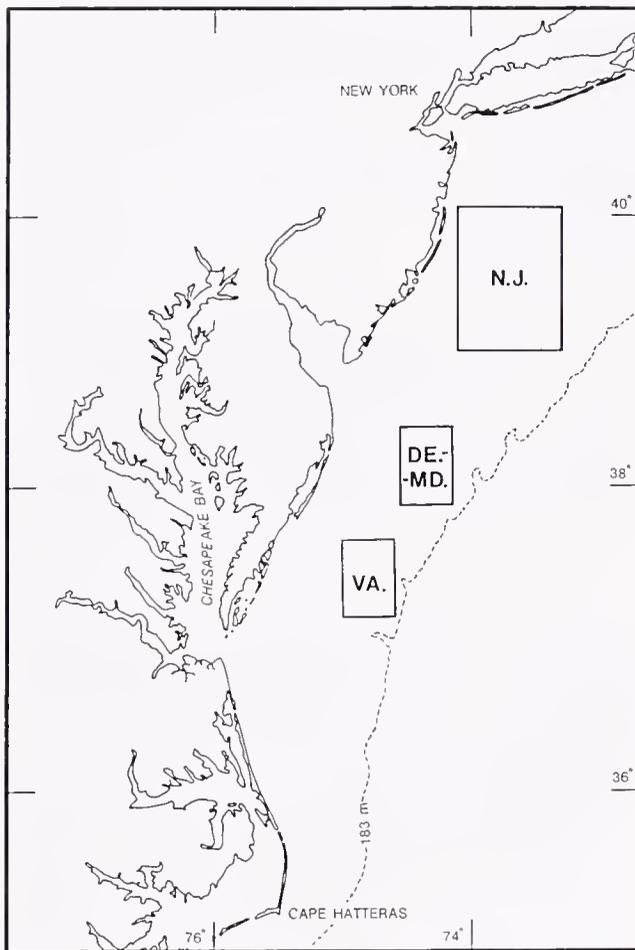


Figure 1. Virginia (VA), Delaware-Maryland (DE-MD), and New Jersey (NJ) sampling areas within the Mid-Atlantic Bight.

determine sex during several months and to define area-specific subpopulations of *Placopecten magellanicus*. The shell-height intervals, although arbitrarily determined, were consistent with National Marine Fisheries Service shell size intervals and indicative of the commercial size distribution. Mean gonad weights for the 4 shell-height groups were subsequently calculated and analyzed to determine temporal patterns in spawning events.

Analysis of the gametogenic cycle and related spawning events was accomplished by several statistical procedures and time-series models. Initially, one-way analysis-of-variance (ANOVA) and Scheffe tests were used to determine temporal similarities and differences in spawning events. Maximum gonad weights during spring and fall months were examined for equality of means between 1987 and 1990. Minimum gonad weights during summer months were similarly examined. Analysis-of-variance, however, does not facilitate examination of periodicity, duration, and relative magnitude of spawning events.

Several time-series methods were used to examine temporal spawning patterns as it has become increasingly apparent in recent years that time-series methods are particularly appropriate for analysis of many fishery-related phenomena (Kirkley et al. 1982, Squires 1986, Fogarty 1988). Time-series methods can be used to specifically determine periodicity, duration, and magnitude of temporally-related events. These methods appear to be particularly well-suited for determining cycles and predicting future values.

Time series data (e.g., means of monthly gonadal weights) are typically comprised of 4 components:

$$GW_t = f(S_t, T_t, C_t, E_t)$$

where  $GW_t$  is monthly mean value of gonadal weight at time  $t$ ,  $S_t$  is a seasonal component,  $T_t$  is a trend component,  $C_t$  is the cyclical component, and  $E_t$  is an error or random component often referred to as "noise." Seasonality and trend indicate periodic fluctuations of constant length and the long-run behavior of a data series. The cyclical component indicates longer-term fluctuations in the data and tends to vary in length and magnitude; it is often characterized as being of an erratic or irregular nature. The 4 components may be additive, multiplicative or some combination of the two.

Normalized seasonal indices (medial average adjusted so that the sum of seasonal indices equals 1200) of monthly mean gonadal weights were calculated, and classical and Census II decomposition methods were used to examine the four components; these methods are described in Makridakis et al. (1983). Decomposition methods do not, however, facilitate modeling or predicting noise or randomness. Additional time-series methods that permit examination of noise were used to further examine the gametogenic cycle and spawning behavior of sea scallops. These other methods included exponential smoothing, Box-Jenkins (1976), and state-space (Akaike 1977, Goodrich 1989).

## RESULTS

Visual examination of mean monthly gonadal weights for the 4 shell-height groups suggests biannual spawning during spring and fall and some consistency in the gametogenic cycle (Figure 2). Gonadal weights typically increased in January; they obtained maximum values between February and April; by June, gonads appeared to be nearly or completely spent. Gonadal weights were typically low between June and August which was indicative of the resting stage identified by Schmitzer et al. (1991). Gonadal weights generally increased again in September and obtained local (a peak or trough but not the most extreme peak or trough for a year) maximum values by October. Gonads were completely or almost completely spent by November.

The mean monthly gonadal weights depicted in Figure 2 indicated global (the most extreme peak or trough for a year) and local maximum values in spring and fall, respectively. Mean values also suggested similarities and differences in spawning events between 1987 and 1990. Changes in gonadal weights during spring 1988 and 1989 were quite similar; weights increased in January and obtained nearly equal maximum values between January and

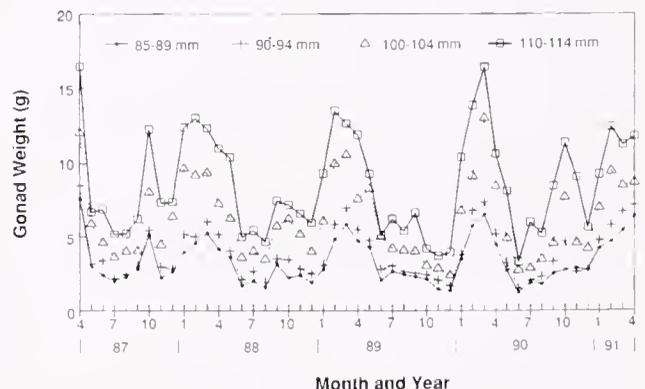


Figure 2. Mean monthly gonadal weights for 4 shell-height intervals of sea scallops in the Mid-Atlantic resource areas, 4/87-4/91.

April. Spawning, as indicated by a large decrease in gonadal weight from a peak value, occurred in June of both years. The fall spawn, however, exhibited a different temporal pattern between 1987 and 1990. In 1988, all 4 shell-height groups appeared to exhibit some level of spawning activity; in 1989, only the 110–114 mm scallops exhibited any fall spawning activity. Spring and fall gonadal patterns for 1987 closely resembled the patterns of 1990 but were quite different than the patterns of 1988 and 1989.

Equality of mean gonadal weights for 100–104 mm scallops over 1987–1990 was rejected by ANOVA and Scheffe tests for all three seasons at the 0.01 level of significance (Table 1). Similar results were obtained for the 3 other shell-size intervals. Maximum mean values for spring were more consistent over the 4 years than they were for fall or summer; differences were detected between 1988 and 1990 and 1989 and 1990. Fall and summer exhibited considerable differences in mean values. Fall pairwise equalities were rejected for all years except 1987 and 1990 and 1988 and 1990. Results of the Scheffe tests suggested that the gonadal cycle did not follow a consistent long-run trend (e.g., maximum mean gonad weights for spring and fall did not consistently increase or decrease from year-to-year).

Examination of the percentage distribution of gonads for 100–104 mm shell height scallops exceeding the mean values also suggested that the spring spawn dominated the fall spawn (Figure 3). Approximately 46% of the sample observations for spring exceeded the mean values, whereas, only 42% of the fall sample observations exceeded the mean values (Table 2). Moreover, maximum spring gonadal weights were consistently higher than maximum fall gonadal weights and exhibited less variation. Similar conclusions were obtained for the other three shell-height groups.

Data plotted in Figure 2 adequately depict seasonal patterns, but normalized seasonal indices provide a more discernible general pattern of seasonality for the gametogenic cycle (Table 3). Indices suggested general seasonal spawns between April and June and October and November. The indices also suggested possible dif-

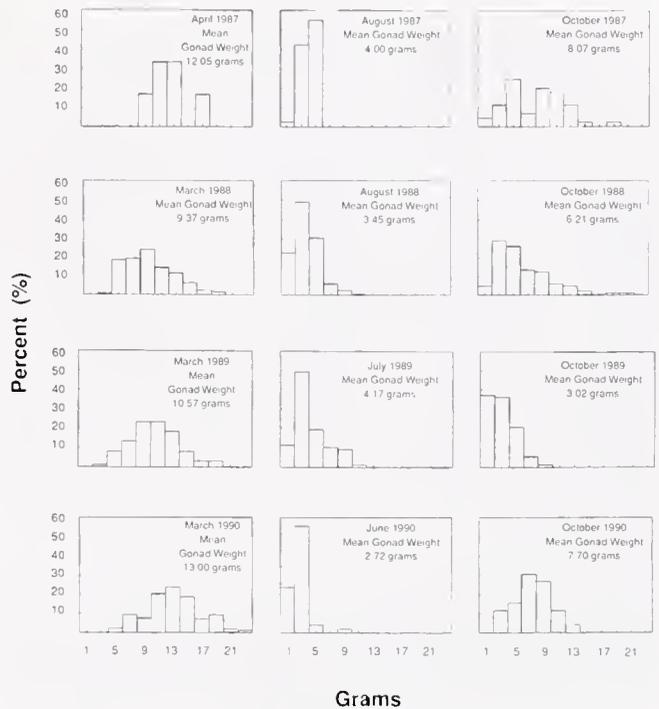


Figure 3. Percent distribution of seasonal maximum and minimum gonadal weights for 100–104 mm shell-height group during annual gametogenic cycle, 1987–1990.

ferences in spawning events of different size scallops. For example, indices of gonadal weights for the 85–89 mm shell-height group suggested a temporally-irregular fall spawn.

Seasonal indices only indicate general seasonal patterns in the gametogenic cycle. They do not indicate variations in the month-

TABLE 1.

Results of analysis of variance (ANOVA) and Scheffe tests of equality of means for 100–104 mm scallops.

Season	Mean Gonad Weight (g)	F-statistic	Pairwise Differences Detected by Scheffe Tests
Spring			
1987	12.05		
1988	9.37	22.32 (3,338) <sup>1</sup>	1988 ≠ 1990 <sup>2</sup>
1989	10.57		1989 ≠ 1990
1990	13.00		
Fall			
1987	8.07		1987 ≠ 1988; 1987 ≠ 1989
1988	6.20	41.14 (3,291)	1988 ≠ 1989
1989	3.02		1989 ≠ 1990
1990	7.70		
Summer			
1987	4.00		1987 ≠ 1990
1988	3.45	11.70 (3,508)	1988 ≠ 1989; 1988 ≠ 1990
1989	4.17		1989 ≠ 1990
1990	2.72		

<sup>1</sup> Numbers in parentheses indicate numerator and denominator degrees of freedom, respectively.

<sup>2</sup> Significant differences detected at the 5% level of significance.

TABLE 2.

Maximum spring and fall and minimum summer mean values of gonadal weights (g) and coefficient of variation for 100–104 mm shell height scallops 1987–1990.

Season and Year	Number of Observations (N)	Mean Gonad Weight (g)	Coefficient of Variation (%)	Number and Percent Exceeding Mean Value	
				N > g	%
Spring:					
1987	6	12.05	23.85	3	50.00
1988	128	9.37	35.99	63	49.22
1989	100	10.57	31.64	51	51.00
1990	108	13.00	28.06	51	47.22
87–90	342	10.92	34.42	158	46.20
Summer:					
1987	40	4.00	24.71	23	57.50
1988	290	3.45	50.15	116	40.00
1989	99	4.17	54.30	38	38.38
1990	83	2.72	44.45	33	39.76
87–90	512	3.51	50.85	191	37.30
Fall:					
1987	45	8.07	47.89	24	53.33
1988	104	6.21	60.88	40	38.46
1989	94	3.02	55.88	33	35.11
1990	52	7.70	33.69	26	50.00
87–90	295	5.74	63.43	123	41.69

TABLE 3.

Seasonal indices of mean values of gonadal weights for 85–89, 90–94, 100–104, and 110–114 mm scallops.

Month	Shell-height Interval (mm)			
	85–89	90–94	100–104	110–114
January	114.4	108.0	119.0	125.2
February	147.5	145.9	157.0	155.9
March	175.4	173.8	163.4	146.7
April	146.5	144.1	124.0	128.8
May	108.6	101.0	101.1	106.7
June	61.8	67.3	69.3	62.2
July	68.0	67.0	65.0	67.0
August	66.3	62.0	62.9	59.3
September	86.2	83.3	76.5	88.0
October	81.9	109.0	117.3	110.0
November	73.1	72.1	76.7	82.0
December	70.5	66.3	67.8	67.5

to-month changes in gonadal weight. Based on decomposition analyses, seasonality explained only 34–37% of the average monthly change in gonadal weight of the 4 shell-height groups (Table 4). Noise or randomness accounted for 57–59% of the average per period change. The trend-cycle was relatively unimportant and responsible for no more than 7% of the average monthly change in weight. Analysis of cyclical factors for June–December 1989 indicated a major change in the gametogenic cycle. Values of cyclical factors for this period were very low which suggested a downward shift in the 1989 fall spawning pattern. Interestingly, the months for cyclical dominance (MCD) factor or time it takes the trend-cycle to dominate the noise was approximately 5 months; an MCD of 5 suggested a need for more extensive analysis of the noise.

Exponential smoothing, Box-Jenkins (1976), and state-space models were thus used to further analyze the possible randomness and underlying dynamics. Standard statistical criteria (Akaike and Schwarz criteria and Ljung-Box) suggested the exponential

TABLE 4.

Percent variation of month-to-month change in mean values of gonadal weights of 85–89, 90–94, 100–104, and 110–114 mm scallops attributed to trend-cycle, seasonality, and random noise.

Shell Height Interval (mm)	Temporal Factor	Percent Explained by	
		Temporal Factor (%)	Factor and Noise (%)
85–89	Trend-cycle	7	
	Seasonality	34	
	Noise	59	
90–94	Trend-cycle	6	
	Seasonality	35	
	Noise	59	
100–104	Trend-cycle	7	
	Seasonality	36	
	Noise	57	
110–114	Trend-cycle	6	
	Seasonality	37	
	Noise	57	

smoothing models did not adequately model the gonadal cycle. The Box-Jenkins models were determined by statistical criteria to better describe the data patterns of gonadal weight for the 4 shell-height groups, but they also failed to adequately model the randomness or noise of the series (Table 5). The models could only explain 70–74% of the monthly variation in mean gonadal weights. Moreover, predictions of 1991 fall gonadal weights were likely incorrect and had large 95% confidence intervals; forecast intervals suggested no spawn and a major spawn.

The Box-Jenkins models for the 100–104 and 110–114 mm shell-height groups both included short-run moving averages of 5. A moving average of order 5 indicated that current values of gonadal weights were explainable by 5 previous forecast errors. The models for the 85–89 and 90–94 mm groups were also similar in structure. Both models had a 1st-order non-seasonal (short-run) and seasonal (long-run) autoregressive component; a moving average component was not supported by analyses of the data.

The estimated state-space models provided more accurate fitted values but likely incorrect predicted values. These models predicted either a minimal fall spawn or no discernible fall spawn for 1991. There are no apparent reasons why there should be no 1991 fall spawn. The approach, therefore, was not further considered in the examination of the gametogenic cycle.

## DISCUSSION

Analyses of mean monthly gonadal weights indicated temporal similarities and differences in the gametogenic cycle of *Placopecten magellanicus* in the Mid-Atlantic region. Over a 4 year period, mean values of gonadal weights suggested biannual spawning but erratic temporal patterns. The temporal patterns for

TABLE 5.

Autoregressive-integrated-moving-average (ARIMA) models used to examine the gonadal cycle of 4 shell-height groupings of Mid-Atlantic sea scallops and summary statistics.

Shell height interval	Transformations To Achieve Stationarity	ARIMA Structure or Form of Model and Summary Statistics <sup>1</sup>
85–89	Logarithmic and 1 seasonal difference	ARIMA (1,0,0) (1,1,0) <sup>12</sup> Ljung-Box: chi-squared (18) = 23.90 R <sup>2</sup> = .72
90–94	Logarithmic and 1 seasonal difference	ARIMA (1,0,0) (1,1,0) <sup>12</sup> Ljung-Box: chi-squared (18) = 17.66 R <sup>2</sup> = .70
100–104	Logarithmic and 1 seasonal difference	ARIMA (1,0,5) (1,1,0) <sup>12</sup> Ljung-Box: chi-squared (18) = 17.64 R <sup>2</sup> = .74
110–114	Logarithmic and 1 seasonal difference	ARIMA (1,0,5) (1,1,0) <sup>12</sup> Ljung-Box: chi-squared (18) = 14.78 R <sup>2</sup> = .70

<sup>1</sup> ARIMA (p,d,q) (P,D,Q)<sup>s</sup> is the shorthand notation for autoregressive-integrated-moving-average models. Lower case letters d and D indicate the short-run or nonseasonal differencing and long-run or seasonal differencing required for stationarity; p and P indicate the nonseasonal and seasonal autoregressive nature of the model; q and Q indicate the nonseasonal and seasonal moving average nature of the model; s indicates the length of seasonality.

fall indicated major fall spawns in 1987 and 1990 and minimal or negligible spawns in 1988 and 1989. In contrast, the temporal patterns for spring were less erratic which is likely indicative of a dominant or major spawning event. Seasonal indices suggested more regularity in spawning events and greater fecundity per individual scallop in the spring; Schmitzer et al. (1991) obtained the latter conclusion via histological analysis.

Although statistical results obtained in this study appear reasonable, they may be biased because of pooling data over sex and area. Pooling may obfuscate patterns of the timing of spawning events by different sexes and in different areas. A small change in the mean value of gonadal weight may erroneously suggest minor spawning events or changes in the reproductive cycle. Alternatively, periods and magnitudes or duration of spawning events determined from analyses of pooled gonadal weights may be imprecise.

Unfortunately, analysis of the gonadal cycle of *Placopecten magellanicus* by sex and area would be costly and difficult. The laboratory work necessary to accurately determine sex would be expensive and time consuming, and area-specific or spatial subpopulations of scallops in the Mid-Atlantic resource area are not defined. Moreover, Schmitzer et al. (1991) found that although a detailed analysis provided a more accurate determination of the reproductive cycle, analysis of gonadal weights pooled over sex and areas was sufficient to determine the general gonadal cycle of sea scallops in the Mid-Atlantic region.

Conclusions about the regularity and dominance of the spring spawn in the Mid-Atlantic resource area differ from previously accepted conclusions about the spawning pattern of Mid-Atlantic and Georges Bank scallops (MacDonald and Thompson 1988, Posgay and Norman 1958, MacKenzie et al. 1978, Robinson et al. 1981). Interestingly, temporal regularity in the fall spawn in the Mid-Atlantic was rejected by analyses of data; the fall spawn appeared to be erratic from year-to-year and nonexistent in 1989.

The absence of an observed 1989 fall spawn may be the result of meteorological events. Hurricane Hugo and several tropical storms occurred in the Mid-Atlantic during October 1989; these storms may have affected normal temporal-related spawning events. Alternatively, the conclusion of no 1989 fall spawn may be associated with possible biases of the data which may have occurred because of the storms. Samples for all weeks of October, however, were obtained, and geographical coverage of the samples remained unchanged relative to samples obtained in other months. Interestingly, analysis of cyclical factors suggested a major downward shift in 1989 fall spawning events (i.e., a temporal intervention).

Unfortunately, extensive time-series analyses of the data failed to adequately characterize short and long-run patterns of the gonadal cycle. General or average seasonal patterns were detected but lacked precision. There was no evidence of a consistent trend in gonadal weight between 1987 and 1991. Cyclical influences, although contributing to variations in weight over the 4 years, were not predictable. At best, the time-series analyses permitted determination of basic patterns of the gonadal cycle; the analyses and models, however, were inadequate for predicting future spawning events.

Failure of the time series approaches to adequately predict future spawning events does not imply, however, that these approaches lack merit. For one thing, the time-series approaches facilitated determination of general seasonal and cyclical patterns and demonstrated an absence of a long-run trend. More important, associated analyses suggested that shocks or stochastic signals of lag 5 substantially affected spawning events. It is not known why stochastic shocks to the system 5 periods ago would affect the current values. This result may be an anomaly caused by the magnitude and duration of the spring cycle (January–May). The time-series analyses also indicated that numerous interventions or innovations affected the spawning pattern of *Placopecten magellanicus* between 1987 and 1991; these interventions may be associated with meteorological events. Last, the time-series approaches indicated a need for longer time series and continued routine monitoring of the gonadal cycle in order to accurately determine the occurrence and temporal pattern of spawning.

Based on the statistical and time-series analyses, the gonadal cycle of sea scallops in the Mid-Atlantic resource area appears to be best characterized as a series of interventions or shocks. There are similarities over time, but for the most part, the timing, duration, and magnitude of spawning events appear to vary widely. These conclusions are not particularly startling given that Naidu (1970) and Rodhouse et al. (1984) demonstrated that environmental factors play a large role in spawning behavior.

Although environmental factors were not considered in this study, results of the time-series analyses indicated that influences other than temporal factors affected spawning. After extracting all temporal related patterns from the gonadal weight data, 25–30% of the variation could not be explained. Considerable attention, thus, needs to be given to examining other factors that may influence the gametogenic cycle and spawning behavior.

The inability to determine the gametogenic cycle and spawning behavior with a high degree of precision may be one reason why it has been difficult to determine an adequate stock-recruitment relationship for *Placopecten magellanicus*. Alternatively, the spring spawn, although consistently occurring, varies widely in magnitude, and the fall spawn varies in timing, magnitude, and duration. Biannual spawning and irregular periodicity of spawning events suggest recruitment may be erratic and occur more than once a year. Empirically determined stock-recruitment relationships that assume single-period, knife-edge recruitment, thus, may be inadequate characterizations of the stock-recruitment relationship of sea scallops in the Mid-Atlantic.

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## FINANCIAL FEASIBILITY AND FARM SYSTEMS IN CULTIVATING *OSTREA EDULIS* LINNAEUS, 1750

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**ABSTRACT** The European oyster, *Ostrea edulis* is being cultivated in the Canadian province of Nova Scotia for the European market. Demand is strong and the European supply is limited and declining, so Nova Scotia could profit from this market. While cultivating oysters is technically viable, there is uncertainty over the financial feasibility of growing them in Nova Scotia, and then air-freighting them to Europe. The financial analysis was examined using two scenarios; a purely commercial operation and a cottage industry. Because of lower capital-labour (K/L) ratios, the latter is more profitable, and support for this activity should be the focus of government policies. If government assistance is required for this infant industry, an effective and equitable policy is subsidised credit.

**KEY WORDS:** *Ostrea edulis*, oyster, cultivation, financial feasibility

### INTRODUCTION

In 1957 the European oyster, *Ostrea edulis* was introduced into Atlantic Canada, but cultivation was handicapped by lack of seed. With the establishment of the hatchery SFT Ventures in Blandford, Nova Scotia, this deficiency was rectified, and now the industry appears to have considerable growth potential. The opportunity lies in an existing market in Europe, which cannot be serviced from traditional sources. Landings of *Ostrea edulis* in Europe have declined dramatically in recent years, and by 1986 were one-fifth those of 1970. The principal cause of the decline was a parasite, *Bonamia*, which has spread throughout the major growing areas of Europe. By 1994 the shortage of locally supplied oysters is projected to be severe, and result in increased prices. With a disease-free stock of *Ostrea edulis*, the industry in Nova Scotia is potentially able to satisfy much of the excess European demand.

While technically viable, the financial feasibility of cultivating *Ostrea edulis* and then shipping them to Europe, remains uncertain. This paper provides some insights. We conclude that cultivating *Ostrea edulis* is not feasible for a specialised operation, but that it is feasible for a cottage-industry.

The initial section of the paper describes the environment of the infant industry in Nova Scotia, while the second section models two farms and analyzes their profitability. The third section examines policies available to governments to promote oyster farming.

#### The Environment

In Atlantic Canada there appears to be one geographical area which is suitable for the cultivation of *Ostrea edulis*. The species requires high salinity and moderate water temperatures (Newkirk 1989). Only in Zone 2 of Figure 1 does this combination appear, and Nova Scotia has been the focus of research and development since the 1970s. Commercial cultivation of *Ostrea edulis* began in 1984 and output reached 10 tonnes by 1987 (Boghen 1989). Initially the industry was handicapped by lack of seed, but the 1988 establishment of a multi-million dollar hatchery at Blandford, Nova Scotia, with Dutch capital, has rectified the problem. In 1989 the hatchery produced 4 million seed of *Ostrea edulis*; this exceeded the total available from all other sources (Bronk et al. 1989). Total capacity is projected at 50 million seed a year.

Two techniques are currently being used for oyster production; suspension and bottom culture. With suspended culture the oysters are grown in nets (Japanese lantern nets) until harvesting begins at the end of year 3. Most are harvested in the fourth year but harvesting continues into year 5 (Newkirk 1989). The quantity of oysters per net is reduced each year, so the number of lines increases from one in the initial year to ten by the grow-out year. This has implications for operating costs, particularly labour and nets. With bottom culture, the oysters are removed from nets at the end of the second year, and left on the bottom. Labour costs are less but mortality rates are higher. In addition appearance and quality suffer.

Not only two techniques, but two systems of cultivation are possible: 1) a commercial farm which maximises profits and relies exclusively on hired labour; 2) a cottage farmer whose principal activity is the capture fisheries. The essential characteristic of a cottage farmer is that he employs no hired labour, thus the labour used is either his own or that of his family. Whether output is sold or consumed on the farm is less important than the dichotomy between labour markets; in practice with oysters much of the output will be sold. Labour on the hobby-farm therefore receives total net output divided by the number of family members; or the value of the average product. The payment of average product is an integral component of family farms (Nakajima 1969). The commercial farm, reliant on hired labour, pays labour a wage equal to the contribution of the last work unit or marginal product. The existence of dual-labour markets has been confirmed in other aquaculture activities e.g. Norwegian salmon farming (Salvenes 1988).

The market for *Ostrea edulis* cultivated in Nova Scotia is primarily Europe. There per capita consumption of shellfish is already high (and growing), ranging from 19.5 kg. per person in Spain to 3.5 in the Netherlands. Net imports of shell fish into the four European countries; Belgium, France, the Netherlands and Spain, reached 150,000 tonnes in 1986. *Ostrea edulis* as a gourmet shellfish is particularly prized, and has a relatively high income elasticity of demand. The European market could absorb between 600,000 to 1.2 million Nova Scotian oysters a year (Bronk et al. 1989). Because of its short shelf life (14 days), *Ostrea edulis*, would have to be air-freighted; costs of this vary according to bulk from \$2.51 per kilo to \$1.72 per kilo. This

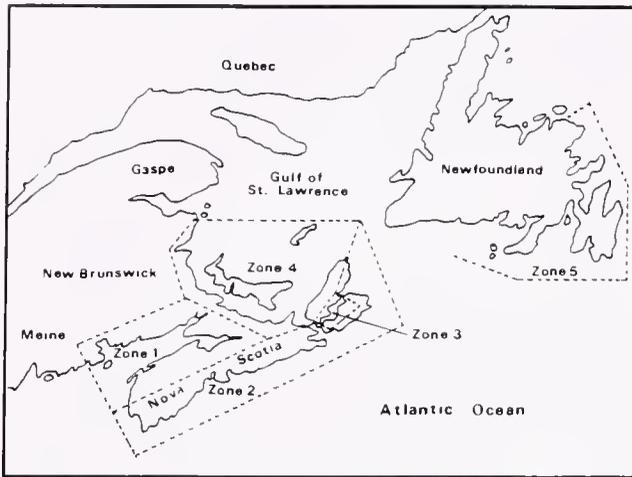


Figure 1. Locations of environmental aquaculture zones 1 (Bay of Fundy), 2 (Atlantic coast of Nova Scotia), 3 (Bras d'Or Lake), 4 (Gulf of St. Lawrence, shallow region), and 5 (Atlantic coast of Newfoundland). (Adapted from Cook et al., 1988.)

transport cost clearly puts a premium on keep operating costs to a minimum.

#### Empirical Analysis

To test the feasibility of oyster cultivation under two different regimes and using two different techniques, a number of assumptions are required. In the first place the paper makes the plausible assumption that the cottage-farmer's land, trucks and boats have zero opportunity cost. The rationale for the assumption is that oyster farming in Atlantic Canada is a cottage industry, considered by fishermen as a source of supplementary income (Lavoie 1989). Oyster farming is ideally suitable as supplementary employment by fishermen because of its seasonal nature. In the harvesting period of oysters, from September onwards, the capture fisheries are almost closed; hence land, boats and trucks are not being used in the fisheries. With this assumption the capital expenditures for cultivating *Ostrea edulis* are given in Table 1.

The second assumption is the retail price of oysters. Presently the wholesale price of *Ostrea edulis* in Nova Scotia is C\$0.35 to

\$0.40 each, and what has been produced has been sold (Newkirk 1989, Muise et al. 1986). After 1994 when the present European supply is reduced, the price is expected to increase sharply. The initial price assumed is \$0.40, although to be conservative results are sensitized for \$0.35. Other assumptions are an annual inflation rate of 5% for both retail prices and inputs, a tax rate of 30% and an investment tax credit of 20%. The time horizon is fifty years, which accords with the longest-lived asset in Table 1, thereby equalising replications (Rashid et al. 1989). The production statistics are given below, with a higher mortality rate assumed for bottom culture, because of predation. The mortality is high, to err on the side of caution (Muise et al. 1986). The farm, whether cottage or commercial is assumed to start with 500,000 spat.

Using Tables 1 and 2 the potential profitability of cultivating *Ostrea edulis* is estimated from net cash flows. The after-tax net operating cash flow is:

$$F_t = (P_t Q_t - OC_t - D_t)(1 - T) + D_t \quad (1)$$

where

- P = retail prices of oysters
- Q = quantity
- OC = operating costs excluding depreciation
- D = tax depreciation (assumed linear)
- T = tax rate

To discount a cost of capital must be determined. The cost of capital is based on the assumption of 50% debt and 50% equity financing. The cost of debt is assumed to be high (at 16%). This is well above the Canadian Prime rate, but incorporates a premium for risk. Also under the Canadian Bank Act underwater livestock cannot be used as collateral against loans. The return on owner's equity is based on the real return for T. bills, an allowance for risk and an illiquidity premium and is 24% (Rashid et al. 1989). The cost of capital is therefore

$$k = 0.5 \times 0.16 (1 - 0.3) + 0.5 \times 0.24$$

which is 17.6%. This is the discount factor used in the Net Present Value (NPV) calculations.

Using the above parameters, Table 3 illustrates the financial forecasts of a cottage-farm using suspension culture.

As Table 3 shows, the cottage-farm has a negative cash flow for its first three years of operation. The cumulative deficit is

TABLE 1.  
Capital expenditures for cultivating *Ostrea edulis*.

Asset	Cost	Life Yrs.	Required Commercial	Hobby
Land/Building	100,000	50	yes	no
Bags	2,694 <sup>1</sup>	7	yes	yes
Boat	10,000	10	yes	no
Truck	17,000	10	yes	no
Scuba Gear	3,500	10	yes	yes
Floats	370 <sup>1</sup>	10	yes	yes
Buoys	70 <sup>1</sup>	10	yes	yes
Rope & hardware	130 <sup>1</sup>	10	yes	yes
Sorter	12,000	20	yes	yes
Work Shack	4,000	25	yes	no
Anchors	70 <sup>1</sup>	25	yes	yes
TOTAL			149,834	18,834

<sup>1</sup> Per line.

Source: William Roderick: A Financial Feasibility Study of Cultivating Oysters: *Ostrea edulis*. MBA Report 1990.

TABLE 2.  
Production statistics of farms with 500,000 spat.

Year	Mortality Rates		Harvest	
	Suspended	Bottom	Suspended	Bottom
1	20%	24%		
2	20%	22%		
3	5%	6%	65,000	60,000
4	5%	6%	195,000	190,000
5	5%	6%	45,000	12,000
Total			305,000	262,000

almost \$90,000. This clearly has major implications for financing. Operating costs are more difficult to finance than fixed costs, because of the absence of collateral. For a commercial operation the negative cash flow is even more constraining. In the first place, because of higher capital expenditures the accumulated cash deficit exceeds \$200,000 for suspended culture (and \$150,000 for bottom culture). In the second place commercial farms lack the supplementary income (from fishing) of cottage farms.

The negative cash flow clearly has an adverse effect on profitability, when discounting is used. Table 4 illustrates the Net Present Value (NPV) of a cottage farm where NPV is the present worth of future net income. With a discount factor of 17.6% the NPV is \$112,348. for bottom culture and \$54,980. for suspension culture. The internal rate of return (for bottom culture) is 29%. Table 5 summarises and includes the commercial farm. As can be seen the commercial farm is not profitable. Even with the lower mortality rate of suspension culture, bottom culture is unprofitable; (with a negative NPV of \$118,773). The evidence suggests that capital expenditures are a major determinant of profitability.

#### Policy Implications

Externalities, which will not be considered by the private entrepreneur, are the principal rationale for government intervention in oyster-farming. These externalities may be detrimental as with pollution, or beneficial as with job creation. There may also be

conflicts over the use of a public good; the waterways, which will require government arbitration. Prince Edward Island for example has zoned its waterways to reduce competition among potential users. Rights to the waterway and to the crop are vital if investment in aquaculture is to occur (Wildsmith 1989).

Beneficial externalities are job creation, foreign exchange earnings and rural development. Oyster cultivation does not compete with commercial landings of (American oyster) since the latter have declined dramatically during the 1980s. As a result, Canada imports approximately 700 tonnes of oysters a year so jobs created in aquaculture represent net gains. Given the indirect effects total employment is difficult to estimate, but oyster farming is more labour intensive than salmon or mussel farming. The cost of a job can be estimated by dividing total capital expenditures (over ten years) by the number of person-years of work; the result ranges from a low of \$14,000 in European oyster cultivation to a high of \$152,000 in Atlantic salmon farming (Ridler and Kabir 1989). So for a dollar invested more employment is generated in oysters than mussels or salmon. In addition to employment, European oyster farming is a source of net foreign exchange for Canada. It is a potential import substitute for the \$3.5 million spent a year on oyster imports; it will also earn foreign exchange revenue from exports to Europe.

Policy options include cash grants, loan guarantees or subsidised credit. All have been used to induce aquaculture in Canada,

TABLE 3.  
Cottage farm using suspension culture.

Year	Revenue R	Operating Costs OC (excl. deprec)	Operating Income OI OI = R - OC	Net Operating Income NOI = OI - D	Tax Liability T	After-tax Net Op. Cash NOI (I - T) + D
1	0	21,351	(21,3501)	(24,437)	0	(21,351)
2	0	39,688	(39,688)	(43,147)	0	(39,688)
3	30,098	56,368	(28,270)	(32,104)	0	(28,270)
4	126,413	78,140	48,272	44,064	0	33,886
5	155,706	84,849	70,857	66,536	0	53,344
6	163,492	86,163	77,329	73,008	13,129	51,026
7	171,666	88,095	83,571	79,250	21,447	50,043
8	180,250	93,018	88,027	83,706	19,555	45,627
9	189,262	98,156	91,106	86,785	21,405	49,944
10	198,725	110,698	88,027	83,706	19,555	45,627
20	323,702	163,354	160,348	145,493	43,648	101,845
30	527,277	261,264	266,013	216,953	65,086	151,867
40	858,879	420,119	438,760	380,642	114,193	266,449
50	1,399,023	609,020	790,003	748,320	224,496	523,824

TABLE 4.

Net present value: cottage farm  
(17.6% discount rate and  $P_1 = \$0.40$ ).

	Bottom	Suspension
After Tax Operating Income	\$239,332.	\$144,026.
Tax Shield from CCA	\$53,793.	\$73,234.
Capital Expenditures	(\$147,930.)	(\$146,229.)
Investment Tax Credits	\$12,662.	\$29,457.
Incremental Net Working Capital	(\$45,509.)	(\$45,509.)
Total NPV	\$112,348.	\$54,980.

but subsidised credit is the most equitable. The burden of cash grants or guaranteed loans (if there is a default) falls exclusively a tax-payers. Subsidising the cost of capital, on the other hand, splits the burden and risk between aquaculturist, bank and taxpayer. The effect on NPV of a (small) reduction in the cost of capital can be significant as Table 6 illustrates. With all the parameters remaining the same, except the cost of capital, Table 6 illustrates how NPV is improved. With a retail price of \$0.40 the NPV of the cottage-farm rises from \$112,348 to \$207,862 for bottom culture; and from \$54,980 to \$118,867 for suspension culture. Given the greater capital in suspension than bottom culture, subsidising its cost clearly has a more pronounced effect. The

TABLE 5.

Net present value: *Ostrea edulis* (17.6% discount rate).

Price	Hobby Farm		Commercial	
	Bottom	Suspension	Bottom	Suspension
\$0.40	\$174,236	\$54,980	(118,773)	(212,540)

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TABLE 6.

NPV with the cost of capital = 15%.

Price	Cottage Farm		Commercial	
	Bottom	Suspension	Bottom	Suspension
\$0.40	\$207,862	\$118,867	(\$41,148)	(\$93,873)

commercial farm remains unprofitable even with a 15% cost of capital; it would have to fall another two percentage points for the commercial farm to break-even.

Whether subsidies should be implemented, and to what extent, is clearly a political decision, but if, as with oysters, the industry is potentially viable, but as an "infant industry" needs assistance, credit subsidies appear to be a viable option. Other options, such as cash credit or loan guarantees may be more risky for tax-payers. Subsidised research and development already occurs through academic research and specialised marine agencies.

## CONCLUSION

The cultivation of *Ostrea edulis* is technically viable and has a large market in Europe. Its financial feasibility is less certain and is determined largely by capital-labour (K/L) ratios. The paper has attempted to demonstrate that cottage-farms, because of their intrinsically lower K/L ratios, are most profitable; and that these should be the focus of government policies. One policy that appears effective is a subsidy on capital costs.

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## ENGINEERING AND BIOLOGICAL STUDIES OF RECONSTRUCTED OYSTER HABITAT

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**ABSTRACT** A laboratory test column for determination of load-bearing capacity of alluvial sediments is described as are methods and procedures to conduct such tests. Two materials, fossil clamshells (*Rangia cuneata* (Sowerby 1831)) and crushed limestone, were evaluated in terms of effectiveness in enhancing load-bearing capacity of dredging-fluidized sediments to recover lost oyster habitat. Using test column-predicted materials requirements, the reliability of the method was demonstrated satisfactorily for a full year on six test plots established on a private oyster lease located in Plaquemines Parish, Louisiana. Both materials were found to be useful for habitat recovery purposes. Similarly, there was little difference between the two materials relative to spat attraction.

**KEY WORDS:** *Crassostrea virginica*, oyster, oyster habitat, settlement, reef recovery, spat

### INTRODUCTION

Strata underlying water bottoms leased for culture of the American oyster, *Crassostrea virginica* (Gmelin 1791) in the estuaries of Louisiana are rich in mineral resources such as petroleum, natural gas, sulfur, and salt deposits, all of which have been extensively exploited. During the past 40 years, mineral resource exploration and production activities have resulted in the dredging of thousands of miles of deep-water access channels across shallow water bodies. Similarly, the marsh in these estuarine areas has been transformed into a patchwork of canals, drilling locations and/or pipeline right-of-ways.

Dredging of oilfield canals to the extent that has occurred in the Louisiana coastal marshes is generally thought to be a contributing factor in the erosion/subsidence loss of this habitat type (Johnson and Gosselink 1982, Craig et al. 1979). Dredging of water bottoms typically results in loss of substrate firmness (load-bearing capacity) such that dredged water bottoms are made unsuited to oyster development. Without remediation, dredging-disturbed bottoms may require six years or longer to achieve reconsolidation to the point that sufficient load-bearing capacity for oyster survival is achieved (Demars et al. 1984). Additionally, silt and other suspended solids introduced into the water column during and subsequent to dredging (or propwashing) may smother existing oyster populations over wide areas relative to the dredging (Hoese and Ancelet 1987) and can cause oyster larvae mortality while in the water column (Carriker 1986). Coating of shells with silt layers also reduces the set of spat and thereby adversely impacts oyster recruitment (Galtssoff 1964).

Cake (1983) developed a Habitat Suitability Index (HSI) model for the American oyster (*Crassostrea virginica*) that includes substrate firmness as one of six major habitat variables used in the model. He reported that optimal substrate firmness for oyster habitat was  $\geq 1$  kg/cm<sup>2</sup>. Standard geotechnical engineering practices involving dredged sediments (Montgomery 1978) include methods for determining substrate and/or spoil bank firmness but typically in much higher quanta, i.e., in terms of several kg/cm<sup>2</sup> relevant only to bearing loads of structures and not the lesser requirements of oyster culture. This paper describes equipment and methodology appropriate to quantitative measurement of very low (e.g., gm/cm<sup>2</sup>) bearing pressures typical of dredged bottom sediments as well as a means of tracking improvements in bearing pressures into the kg/cm<sup>2</sup> range that result from the addition of surface layers of

clamshell (*Rangia cuneata*) as compared to the use of No. 57 crushed limestone.

### MATERIALS AND METHODS

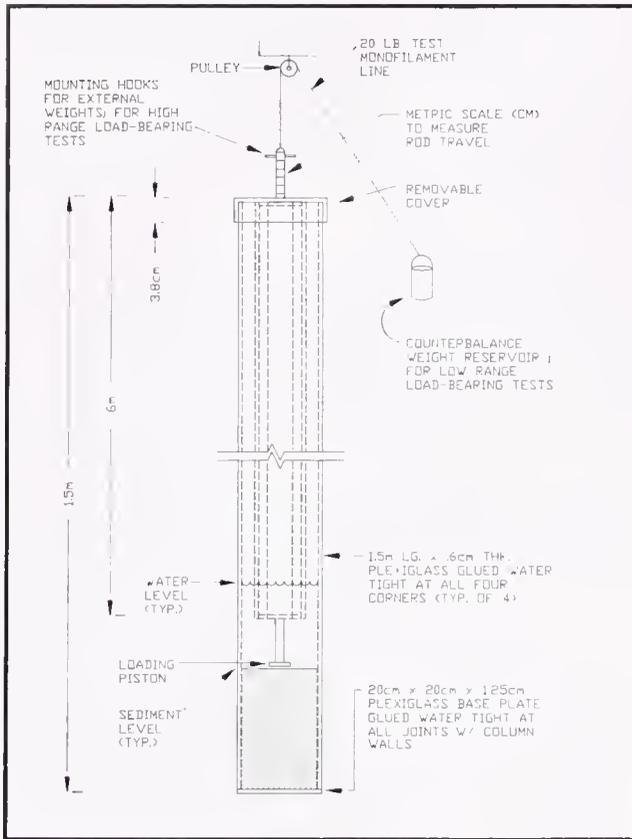
#### Load-Bearing Capacity Studies

A modified settling column device (Brodtmann et al. 1987), illustrated in Figure 1 and referred to as a load-bearing test column (LBTC) was fabricated and equipped with a loadable piston which provided a means of determining the bearing pressure of settled sediment slurries with very low load-bearing capacity (0 to  $<0.1$  kg/cm<sup>2</sup>). Two pistons were employed, one with a 5.08 cm  $\times$  5.08 cm pressure plate and one with a 2.54 cm  $\times$  2.54 cm pressure plate. The former was used for very soft, unenhanced sediments (load-bearing capacity  $<0.05$  kg/cm<sup>2</sup>), whereas the latter was used for "restored" sediments with much higher load-bearing capacities ( $\geq 1$  kg/cm<sup>2</sup>) required to achieve failure.

Sediment characteristics in the study area were reported by Barrett (1971) to be generally silt/clay. Sediment samples were obtained monthly on four different occasions by ponar dredge from the water bottoms of Bay Lanaux in an area known to have been dredged within the last five years. Bay Lanaux is a small brackish bay located in Plaquemines Parish, Louisiana about 130 km south of New Orleans. The sediments were placed in ice chests and transported wet to the lab. Sufficient quantities of bay water were obtained at the same time to enable slurry preparation for each test thereby maintaining an "ambient" salinity relationship for each experiment.

In an adaption of the methods reported by Thackston et al. (1984) sediment slurries prepared by homogenizing 12 L of wet silty/clayey sediments with 8 L of bay water were prepared and then poured into the LBTC. A 1.6 cm diameter air hose supplied with an airflow of 0.2 to 0.4 m<sup>3</sup>/min. at 414 kPa was then lowered into the column to ensure a homogeneous suspension by vigorous air-lift mixing for 2 minutes.

Zone settling measurements (Palermo 1978) were then made over a period of several days until such time as the sediment settling/compaction rate had decreased to 1 mm or less per 24 hours. The LBTC piston fitted with a 5.08 cm (square) pressure plate then was placed into the column while counterbalanced by an external weight reservoir such that no pressure was applied to the surface of the recently consolidated sediment. Small increments of



**Figure 1.** Illustration of the load-bearing test column (LBTC) used in laboratory determinations of materials requirements for sediment surface enhancement.

weights (30 to 60 gms) were removed from the counterbalance weight reservoir thereby applying approximately the same increment of pressure onto the sediment at the piston plate. The increments of weight removed were recorded. The piston plate was allowed to settle for 15 minutes after each increment of weight was removed. Piston height was recorded just before removal of the next weight increment. The weight of the piston (530 gms) alone without any counterbalance weights was found to exert a pressure of about  $0.02 \text{ kg/cm}^2$ .

In classical engineering terms, load-bearing capacity of a sediment or soil is defined as the pressure at which failure occurs (Sowers 1979). For the purpose of this study, all tests were considered to have reached failure when piston settlement into the recently consolidated sediment met or exceeded 4 cm. This is the point at which an oyster sinking into the sediment would die.

In 1987 (prior to the start of this work) six side-by-side recompaction/load-bearing tests were run using sediment and water acquired from the same area of Bay Lanau. The procedures and quantities used were identical to those reported here for the initial, unenhanced recompaction tests. However, no attempts were made at that time to enhance load-bearing capacity of recompacted sediments as is reported here.

In later experiments, each conducted after sediment settling to maximum consolidation, improvement in load-bearing capacity resulting from the application of a layer of clamshell was determined. Shell layers of approximately 2.5 cm, 5 cm, 10 cm, 15 cm, 20 cm, and 25 cm thickness were tested. Sufficient quantities of clamshells were measured volumetrically to achieve the desired

shell-layer thickness. The shells were then slowly poured into the test columns with every effort made to ensure a uniform layer. The same parameters and procedures were also employed for tests with the No. 57 crushed limestone. Clamshells used in this study were from the species *Rangia cuneata*. The shells were obtained from a commercial dredging operation from fossil deposits in Lake Pontchartrain, Louisiana. Shell height ranged from 15 to 45 mm. Limestone used was No. 57 grade Kentucky limestone obtained from a local materials supplier.

Material layers (shells or limestone) of 2.5 and 5 cm were tested using a piston with a 5.08 cm (square) pressure plate whereas material layers of 10 cm or more were tested with a 2.54 cm (square) pressure plate. In the latter cases the weight of the piston alone was insufficient to cause settlement. Therefore, lead weights were attached to the upper end of the piston shaft to incrementally increase piston pressure. The weight increments were recorded as added and the height of the piston was recorded following settlement after each weight addition. Additional weight increments were added until the piston settlement had reached or exceeded the 4 cm limit. All consolidation rate tests and bearing pressure tests (with and without shells or limestone) were conducted in triplicate. Each of the replicates for a given enhancement material thickness were conducted simultaneously (three columns for each material) using fresh sediment and bay water in each column replicate. After load-bearing capacity enhancement levels had been determined sediments and bay water were discarded. Enhancement materials (clamshells and crushed limestone) were thoroughly washed on a screen and recovered for later reuse.

The relationship between the increase in load-bearing capacity as a function of increasing thickness of the surface enhancement layer was examined for both materials by means of cubic regression analysis which appeared to provide the best fit of the raw data as compared to that provided by various other forms of regression analysis attempted. These statistical analyses were kindly provided by David Colby (NMFS), the study grant's Contracting Officer's Technical Representative (COTR).

#### Field Demonstration

Six  $3 \text{ m} \times 3 \text{ m}$  demonstration plots were constructed on the soft sediment of Bay Lanau. Approximately,  $1.4 \text{ m}^3$  of sediment surface enhancement materials were spread by hand on each of the test plots. Water depths in the study area ranged from about 0.75 m to 1.5 m which prevented visual observation of the test plots. Three of the demonstration plots (Nos. 1, 3, 5) were treated with about 15 cm thick layers of No. 57 crushed limestone and the other three (Nos. 2, 4, 6) were treated with the same thickness of clamshells. This thickness of materials was selected on the basis that it was the mean (and median) thickness used in the in-vitro column tests. Further, the optimal load-bearing capacity value of  $1.0 \text{ kg/cm}^2$  identified in the Oyster Habitat Suitability Index (Cake 1983) appeared to be overly conservative and thus too costly to achieve. Therefore, the demonstration plots were designed to achieve only about 60 percent of the index value.

Approximately one week after installation of the test plots a land survey of the plots was conducted to determine the starting elevation of the respective plots. Plot elevations were determined one year later shortly before the study was concluded.

On November 30, 1988, 15 sacks of oysters (approximately 3,000 live oysters), harvested, culled and sacked the same morning, were transported to the study area. About 500 mature, live

oysters were then placed on each of the test plots to determine the ability of the "reconstructed" water bottoms to support oysters. All specimens were examined and the shell height of each was recorded prior to "planting" on the test plots. Oysters used to populate the test plots ranged in size (shell height) from about 60 mm to 150 mm with the majority of the specimens' shell height falling within the narrower range of 60 mm to 90 mm.

From December 24 through December 26, 1989 a very unusual, hard freeze occurred over south Louisiana that killed 60 to 70 percent of the oysters that were placed on the test plots. Therefore, beginning on January 26, 1990 all remaining live oysters were picked up from the test plots, dead specimens were discarded and the test plots were replanted with the remaining live oysters along with new oysters obtained from a commercial oyster house. As before, each oyster's shell height was measured and recorded prior to "planting" on the respective test plots.

Test plot No. 2 was abandoned during the first three months after it was overrun by an unknown vessel. The five remaining test plots were monitored on May 15, August 20, and finally, on December 26, 1990. In each monitoring event oysters were removed by hand, tallied as to status (live or dead), measured (shell height), and carefully replaced on the test plots from which they had been collected. Dead specimens were discarded. No attempt was made to determine the cause of mortalities observed. However, burial of oysters was not detected at any time as evidenced by the fact that no specimen (dead or alive) on any of the test plots were observed to have blackened valves.

This study was not designed or controlled to contrast the two sediment enhancement materials relative to spat attraction. Nevertheless, during the first two population monitoring events, spat found on the remediation materials and on the oysters introduced to the test plots also were noted and tallied. All specimens retrieved for tallying were obtained by touch as the sampler's hands were moved slowly over the test plots' surfaces. After the oysters were removed from the respective plots at the end of the study (December, 1990) the first 2 to 3 cm of enhancement material was scraped up with shovels to be examined for spat. Spat counts for those spat that set on shells of the introduced oyster populations are not reported for that monitoring event. That is, only those spat found to have set on either the clamshells or on the crushed limestone media were reported.

## RESULTS

### Load-Bearing Capacity Studies

Load-bearing capacity data for recompacted sediment tests conducted prior to this work were found to be almost identical to current data (Table 1). Load-bearing data (Table 2) for sediment

TABLE 1.

Load-bearing capacity values (LBC) for prior and current studies with settled sediment from Bay Lanoux (no surface enhancement used).

	Mean LBC of Sediment (kg/cm <sup>2</sup> , 6 reps.)	Standard Deviation
1987 data	0.011	0.0014
This study	0.011	0.0014
All data pooled	0.011	0.0014

TABLE 2.

Mean load-bearing capy. (LBC) for lab tests of settled sediment with surface layer enhancement using clamshells or limestone.

	Mean LBC (kg/cm <sup>2</sup> )	Standard Deviation
Clamshells (5 cm)	0.196	0.022
Limestone (5 cm)	0.186	0.011
Clamshells (10 cm)	0.291	0.001
Limestone (10 cm)	0.252	0.009
Clamshells (15 cm)	0.733	0.157
Limestone (15 cm)	0.764	0.111
Clamshells (20 cm)	0.979	0.312
Limestone (20 cm)	1.020	0.332
Clamshells (25 cm)	0.869	0.013
Limestone (25 cm)	1.040	0.025

columns with various thicknesses of surface enhancement added were utilized in a statistical analysis (Student's t test for equality of means, modified for small samples where  $n < 30$ , (Bailey 1976)) to evaluate the differences in surface enhancement effectiveness between clamshells versus limestone. The observed differences were not statistically significant ( $p = 0.01$ ). The t-test was used instead of other statistical analyses because of the very small sample sizes involved (i.e.,  $N_1 = N_2 = 3$ ).

The cubic regression model employed to examine the relationship between load-bearing capacity and thickness of surface enhancement layers applied was as follows.

$$LBC = a + b_1(T) + b_2(T^2) + b_3(T^3)$$

where:

LBC = load-bearing capacity, kg · cm<sup>-2</sup>

a = the intercept

$b_1$  = component of slope attributable to thickness

$b_2$  = component of slope attributable to square of thickness

$b_3$  = component of slope attributable to cube of thickness

T = thickness of enhancement layer

An ANOVA test of the regression and deviations from the regression showed the model to be significant ( $F = 52.239$ ,  $p = 0.0001$ ). Almost 86 percent of the variation in load-bearing capacity was accounted for by the model ( $R^2 = 0.8577$ ). A plot of the cubic regression model predicted load-bearing test column data is shown in Figure 2 as are actual column test data.

The unexpected drop-off in load-bearing capacity shown for the thickest layer of both materials tested (25 cm) is believed to be an anomaly that is solely a function of experimental design and does not indicate true load-bearing capacity at that point. It is suggested that the 25 cm thick layer had sufficient weight to compress the sediment below such that the entire mass moved down. Measurements of piston rod travel were made at the top of the column and, as the mass of sediment and enhancement layer compacted, the rod moved down to the 4 cm failure level. Yet, the piston pressure plate probably had not actually penetrated 4 cm into the enhancement layer.

### Field Demonstration Plots

Based on land survey elevation data, all of the test plots (plot No. 2 excluded) were found to be stable having remained level and at essentially the same elevation relative to MSL datum one year

TABLE 3.

Summary of oyster population data for three limestone (L) and two clamshell (C) test plots. S.E.M. values shown in parentheses.

	January 1990		May 1990		August 1990		December 1990	
	L	C	L	C	L	C	L	C
Total no. live	1510	981	1688	971	2943	1696	1743	1170
Mean no. live	503 (14)	490 (28)	563 (42)	485 (30)	981 (192)	848 (164)	581 (183)	585 (52)
Percent dead	0	0	8	6	5	7	8	6
Mean no. spat (<45mm)	2 (1)	4 (3)	33 (18)	42 (21)	37 (20)	40 (19)	15 (6)	11 (5)

after placement. The very firm plots easily supported the weight of two 80 to 90 kg samplers during each population monitoring event. Thus, it is likely that the recovery technique likely would be effective even if thinner layers of limestone, e.g., 5 to 8 cm, were to be used for oyster habitat recovery.

Oyster abundance data for the respective test plots are summarized, according to surface material used, in Table 3. The total number of live oysters shown for the clamshells plots (981) reflect the loss of test plot No. 2. The population counts for both materials given for the December, 1990 monitoring event reflect the loss of oysters apparently stolen from test plots Nos. 5 and 6 sometime after August, 1990. This assumption was made on the basis that sufficient dead oysters were not found on those two plots to account for the total drop in number of live oysters found.

For the three monitoring events after the January startup, the difference in spat counts between clamshells and limestone are minimal. An average of 365 spat and young oysters were found on the limestone plots and an average of 280 spat/young oysters were found on the clamshell plots nine months after the test plots were repopulated. These data suggest a limestone to clamshell spat recruitment ratio of about 1.3 to 1.0. Chatry et al. (1986) reported a limestone to clamshell spat set ratio of 2.1:1.0. Broadhurst III (1990) effectively confirmed the latter spat recruitment ratio in laboratory and field studies. The lower ratio reported here likely reflects only the experimental design which did not place emphasis on determination of spat recruitment throughout the course of the study. Other factors, including, for example, site hydrographic characteristics, would have to be taken into consideration in order to properly segregate the relative merits of the two enhancement materials in terms of spat recruitment effectiveness.

## DISCUSSION

For unenhanced sediments tests the load-bearing test column and methods were found to be reproducible with almost no variation seen among test results obtained in 1987 as compared to the tests reported here. Similarly, little variation was seen among replicates in load-bearing capacity enhancement tests run with clamshells and with limestones.

Chatry et al. (1986) and Broadhurst III (1990) found these two materials to be effective in terms of spat recruitment, with similar spat count ratios, i.e., 2.1:1 and 1.9:1, respectively. The long term (9 months) comparison provided by this work showed that ratio under normal field conditions to be more on the order of 1.3:1. Moreover, this work is the first to report the equal effectiveness of the two materials in terms of habitat recovery applications.

The latter finding is a fortuitous one in the sense that, during the course of the field demonstration, the Louisiana Department of Environmental Quality permanently halted all clamshell dredging operations in the State. With the source of clamshells thus curtailed, the findings pertaining to effectiveness of crushed limestone for oyster habitat recovery has increased relevance.

The work reported here employed only a single sediment type (silt/clay) throughout the course of the column testing phase. From the standpoint of use by oyster habitat resource managers over a wide geographic area, it is indicated that additional tests employing other sediment types (e.g., sand/silt) should be conducted to determine post-dredging recovery requirements. With attention being given to sediment types involved, the equipment and methods reported here can be employed by those resource managers to better utilize limited culch material resources for shell plants, and to determine mitigation requirements for activities in coastal waters that may cause the destruction or diminution of oyster habitat.

Similarly, additional field studies are indicated to define the lower load-bearing capacity limits of effective habitat recovery as the quantities of limestone (15 cm thick) used in this study appear to have provided more load-bearing capacity on the test plots than is necessary for oyster culture.

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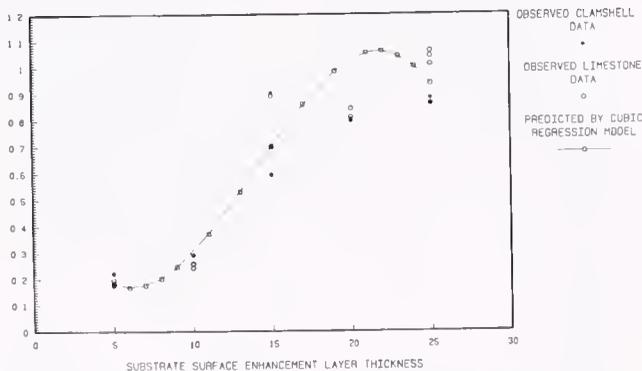


Figure 2. Plot of cubic regression model of LBTC data as compared to raw data for clamshells and for limestone surface enhancement tests.

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## ALTERNATIVES TO CLAMSHELL AS CULTCH FOR OYSTERS, AND THE USE OF GYPSUM FOR THE PRODUCTION OF CULTCHLESS OYSTERS

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**ABSTRACT** The effectiveness of clamshell (*Rangia cuneata*) (Gray 1831), limestone, gravel, and concrete as substrate for settlement of the American oyster, *Crassostrea virginica* (Gmelin 1791), was compared in field and laboratory experiments. A method to produce cultchless oysters using gypsum was also investigated. Oyster set on limestone in the field experiment was significantly ( $p < 0.05$ ) greater than on clamshell; limestone, clamshell, and concrete attracted significantly more spat than did gravel. In the laboratory experiment, oyster set on clamshell and limestone was significantly greater than on concrete, and all substrates were significantly superior to gravel. Limestone should prove to be an economically feasible, biologically acceptable, and environmentally benign alternative to clamshell. Set on gypsum matched that on clamshell in hatchery experiments. Larvae metamorphosed and subsequently detached from dissolving gypsum, thus providing cultchless spat.

**KEY WORDS:** *Crassostrea virginica*, *Rangia cuneata*, oysters, clamshell, cultch, cultchless oysters, gypsum

### INTRODUCTION

Productive oyster grounds in Louisiana are being rapidly diminished by natural and manmade processes (Soniata and Dugas 1988). Increased salinities and sewage problems have shifted the productive zone away from historically productive reefs into areas that generally lack suitable substrate (Chatry et al. 1986).

The Louisiana Department of Wildlife and Fisheries deposits large amounts of clamshell (*Rangia cuneata*) on seed grounds as cultch for oyster larvae. Clamshell has been the preferred material since the mid 1960's due to low cost, availability, and suitability. Areas with firm, stable bottoms and favorable salinities are chosen as cultch plant sites (Dugas 1988). The main source of clamshell has been from vast deposits in Lake Pontchartrain. Dredging for clamshell, however, was recently banned in the lake due to adverse ecological impacts. Alternative cultch materials must now be considered in light of the uncertain availability and the escalating cost of clamshell.

Several alternatives to clamshell have previously been investigated. Butler (1955) tested Plexiglas, frosted glass, and cement board, whereas Shaw (1967) examined setting on asbestos-plate collectors. Other materials have included stones, bamboo, tiles, tarred ropes, paper, wood, ceramics, and many plastics. Tiles and plastics with a lime coating have also been examined (Quayle and Clark 1971). Asbestos-cement plates were used by Kennedy (1980), Hidu et al. (1981) tested polished granite and polished marble, and Mann et al. (1990) examined expanded shale and tire chips. Crushed oyster shells from shucking houses are suitable, but expensive and limited in availability (Chatry et al. 1986). Reef oyster shells were found to be suitable (St. Amant 1959), but the adverse effects of extensive reef dredging limit their use (Bouma 1976). Limestone was found to catch about twice as many larvae as clamshell (Chatry et al. 1986), but limestone is 40% more expensive than clamshell by volume (as of May 1990). Coal ash is now being investigated, and preliminary results from a Texas

study indicate that it may prove to be an acceptable alternative (Ray, personal communication).

Oyster larvae will set upon a variety of hard surfaces; however, there appears to be some property associated with a molluscan shell, perhaps calcium carbonate, which seems to enhance a cultch's attractiveness to setting larvae (Hidu et al. 1975). Oyster larvae are induced to initiate settlement behavior upon the detection of bacteria-associated chemicals (Coon et al. 1985). Larvae will, during their characteristic crawling behavior across the substrates, respond to factors such as light, texture, and chemical cues which determine the suitability of the substrate. The larvae will resume swimming and settle elsewhere if the substrate is unacceptable (Coon et al. 1985). The presence of spat on cultch also seems to stimulate setting (Crisp 1967, Hidu and Haskin 1971, Keck et al. 1971), and there may be some substance secreted by spat which leads to this gregarious induction (Crisp 1967, Hidu 1969).

Suitable substrates must be economically feasible, biologically acceptable, and environmentally benign. Economic concerns include costs of materials, transportation, and planting. Limestone, crushed road bed (concrete with some asphalt), and gravel were among the available materials possibly meeting these criteria.

Gypsum also met the requirements of being available and inexpensive; its solubility in seawater, however, prevents its use as a substitute for clamshell in the field. However, this characteristic might allow gypsum to be used to produce cultchless oysters in a hatchery situation. Cultchless oysters have several advantages over attached oysters, including uniform shape, ease of shipping and shucking, and elimination of costs associated with cultch materials (Dupuy and Rivkin 1972). Cultchless oysters are also easily manipulated and measured in laboratory studies (Coon et al. 1986). Previously published methods for producing cultchless spat include dislodging newly set spat using specialized equipment, such as flexible Mylar sheets, water jets (Dupuy and Rivkin 1972), or lead sheets (Budge and Freeborn 1970). Other procedures use small calcium carbonate particles, including tropical beach sand, foraminiferal sand, marble chips, and mollusk shell chips as cultch to produce "semi-cultchless" spat (Hidu et al. 1981). Coon et al.

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(1986) used epinephrine to induce metamorphosis without attachment, producing cultchless spat. While these authors report induction rates of greater than 80%, other researchers report difficulties in reaching similar results (Ray, personal communication). Another method uses a specialized substrate of compressed Portland cement, lime, and gypsum which decomposes (Quayle and Clark 1971). The main drawback to this method is the cost of producing a specialized substrate. Gypsum, without any of these disadvantages, was therefore considered for use as cultch in the hatchery production of cultchless oyster spat.

The purpose of this study was to test different cultch materials for use in producing attached and cultchless oysters. Laboratory experiments were performed to develop a method for producing cultchless spat using gypsum. Field and laboratory experiments were conducted to test the relative spat catching abilities of different cultch materials for use as alternatives to clamshell.

## MATERIALS AND METHODS

### Cultch Experiments

#### Field Experiment

Clamshell, concrete, gravel, and limestone were obtained from New Orleans area materials companies and tested for their ability to catch spat. Clamshell was dredged from Lake Pontchartrain, crushed road bed (mostly concrete) was taken from Louisiana highways, gravel was dredged from the Pearl River, and limestone was quarried in the Tennessee valley. Clamshells were whole single valves, ranging in size from 2–5 cm in diameter. Limestone pieces were roughly rectangular in shape with multiple faces, ranging in size from 1 × 2–3 × 6 cm. Chunks of crushed concrete ranged in size from 2 × 2–5 × 7 cm. Gravel was rectangular to oval, ranging in size from 1 × 1–3 × 5 cm. Open, plastic coated wire mesh trays (50 × 50 cm; 1.27 × 1.54 cm mesh) filled with 5 cm of substrate were placed in random (Hollander and Wolf 1973; 40 replicates for each substrate) subplots in a 0.25 hectare experimental pond at Grand Terre, Louisiana. Water from lower Barataria Bay, which served as a source of larvae, was pumped into the pond. The temperature (mercury thermometer), salinity (refractometer; Behrens 1965), dissolved oxygen (Azide Winkler modification; American Public Health Association 1971), and flow (volume/time) of the water were monitored weekly, as were test trays of each substrate. After 12 weeks, the trays were removed, spat were counted, and the volume of material in each tray was measured by displacement. (Spat volume represented no more than 0.16% of total cultch volume.) The number of spat per subplot was converted to spat per liter and spat per cubic yard of material for statistical and comparative purposes. Oyster settlement as a function of substrate was examined using non-parametric, one-way analysis of variance (NPAR1WAY; SAS Institute 1979).

#### Benthic Sinkage Experiment

Relative sinkage of each substrate was tested and recorded. Mud (12 l) and seawater (8 l, 15 ppt) from a Grand Terre pond were placed together into 5 Plexiglas containers each measuring 1.5 m high by 20 cm square. Each mixture was homogenized by circulating the mixture with a 1.6 cm air hose (60 psig) for 90 seconds. Sediment:water interface levels were measured daily un-

til a variation of less than a 1 mm per 24 hours was reached (Brodthmann unpubl.). The mud/water interface of the control was measured to insure that no independent settling of the mud had taken place. Each substrate was placed in a container to a height of 3 cm above the interface. Sufficient substrate was added daily to maintain this constant height. Weight and volume measurements of each substrate were taken when settling slowed to less than 1 mm per day. The volume of material needed to cover one acre to a height of 3 cm after accounting for benthic sinkage was determined from these measurements. By setting the sinkage value of clamshell to 1.0, relative sinkage coefficients were calculated. Particle size of the sediment was determined according to the method of Folk (1980).

#### Cost Analysis

Material and transportation costs (obtained from area materials companies) were combined with information on sinkage and spat catch to determine the relative cost effectiveness of the cultches. Dry weight, water volume displacement, and surface area (area of coverage with aluminum foil) were determined for one dry liter of each substrate. Material cost per acre was calculated from delivered cost and the amount needed to cover an acre. Sinkage into the bottom was taken into account by multiplying cost per acre by the appropriate sinkage coefficient. Cost per spat was calculated by dividing mean values for spat per yd<sup>3</sup> by cost of delivered materials.

#### Laboratory Experiment

A replication of the field experiment was conducted at the Texas A&M oyster hatchery (Galveston, Texas) under more controlled conditions and with a greater concentration of larvae. One liter of each cultch was randomly assigned to each of 21 subplots (Hollander and Wolf 1973), and placed in a 1.8 m diameter fiberglass tank and filled to a depth of 0.3 m with seawater. In contrast to the field experiment, there was no spacing between subplots.

Larvae were cultured and set according to methods of Dupuy et al. (1978). Ripe oysters were induced to spawn by elevating the water temperature to 29–30°C. Spawning occurred in separate containers, after which time sperm and eggs were pooled. After one hour, fertilized eggs were counted and stocked (16 eggs/ml) into a 250 l fiberglass tank. The tank was drained every two days; after the first and second drainings, larval concentrations were reduced to 8 larvae/ml and 4 larvae/ml, respectively. The larvae were fed Tahitian strain *Isochrysis galbana* at the rate of 1000 cells/larvae/day. Larvae reached the eyed-stage after about 10 days. Eyed larvae were held in refrigerated moist mesh bags to insure vitality prior to setting. Approximately 400,000 larvae were introduced into the setting tank.

Live spat were counted after 2 weeks and data were converted to spat per cm<sup>2</sup> of cultch for comparison. Oyster settlement as a function of substrate was examined using non-parametric, one-way analysis of variance (NPAR1WAY; SAS Institute 1979).

#### Cultchless Experiments

#### Solubility Experiments

The rate of loss of gypsum (calcium sulphate), was measured in two flowing and two static ambient seawater systems at the

TABLE 1.

Oyster set on clamshell, limestone, gravel, and concrete in Grand Terre, Louisiana field experiment as recorded in August 1990.

	Clamshell	Limestone	Gravel	Concrete
Average spat/subplot .....	1.7	3.8	0.2	3.1
Total spat/total subplots .....	68	152	8	124
Volume (l) of cultch/subplot .....	8.0	9.2	5.6	10.6
Number of spat/l .....	0.22	0.41	0.05	0.29
—ranges	0–0.64	0–1.56	0–0.56	0–0.68
—C.V.	0.835	0.982	2.304	0.584
Number of spat/yd <sup>3</sup> .....	4.7	8.7	1.1	6.2
Average spat/cm <sup>2</sup> .....	0.08	0.20	0.02	0.20
—ranges	0–0.24	0–0.77	0–0.24	0–0.47
—C.V.	0.859	0.989	2.492	0.588

LUMCON marine laboratory (Cocodrie, LA). The texture and shape of the rocks varied, most being rough and rectangular, ranging in size from 1 × 1–9 × 16 cm. Thirty rocks were rinsed, weighed, and placed linearly in each of two 0.4 × 2.2 m trays. Ambient seawater (6–24 ppt, 15.6–22.6°C, 30 µm filter) was pumped into the flowing system (10 cm depth) at a rate of six liters per minute from 16 November 1989 to 22 February 1990. Rocks were removed once a week, gently shaken to remove silt, air dried for ten minutes, and weighed. Percent weight loss per week was calculated for each system.

#### Hatchery Experiments

Gypsum's attractiveness to setting larvae was examined to determine if it could be used to produce cultchless oysters. Larvae were cultured and set according to methods outlined above (Dupuy et al. 1978); approximately 60,000 eyed larvae were introduced into a 1.8 m diameter fiberglass setting tank. Water remained static until setting was completed (48 hours), at which time spat were counted. Water was then pumped into the tank to hasten dissolution, and the remaining gypsum was removed once spat became detached.

The number of spat per rock or shell was converted to the number of spat per cm<sup>2</sup> using surface area measurements. Oyster settlement as a function of substrate was examined using analysis of variance (NPAR1WAY; SAS Institute 1979).

## RESULTS

#### Cultch Experiments

##### Field Experiment

Water temperature in the Grand Terre pond ranged from 26.6° to 32.8°C (May to August, 1990), with a mean of 30.1°C. Water

flow ranged from 0 to 208 l per minute, whereas salinity ranged from 12 to 17 ppt, with a mean value of about 15 ppt. Dissolved oxygen levels ranged from 5.8 to 10.0 ppm. Organisms other than oysters which became sequentially attached to cultch materials included green algae, mussels, barnacles, and bryozoans. There appeared to be more fouling on the concrete and limestone, with minimal fouling on the gravel.

The means (and ranges) of spat per liter were 0.22 (range = 0–0.64; C.V. = 0.84) for clamshell, 0.41 (range = 0–1.56; C.V. = 0.98) for limestone, 0.29 (range = 0–0.68; C.V. = 0.58) for concrete, and 0.05 (range = 0–0.56; C.V. = 2.30) for gravel (Table 1). Analysis of variance of spat per liter of cultch data shows that all possible two-way comparisons of cultches were significantly ( $P < 0.05$ ) different, except clamshell versus concrete and concrete versus limestone. Limestone had a significantly greater ability to attract spat than did clamshell or gravel. Gravel was found to be significantly less apt to attract spat than each of the other cultches.

#### Benthic Sinkage Experiment

Clamshell was the lightest cultch with the greatest surface area, and consequently sank the least (Table 2). Limestone, heavier than concrete and with a greater surface area, had a sinkage coefficient of 2.5, requiring 2.5 times the volume of clamshell to cover the same area. Gravel, heavier than concrete but with a greater surface area, had a sinkage coefficient of 3.0. The sinkage coefficient for concrete was 3.2. Results of the particle size analysis showed that the sediment used was 93.1% sand, 3.5% silt, and 3.4% clay.

#### Cost Analysis

A cost analysis shows limestone to be 1.2, 1.4, and 7.3 times more cost effective in attracting spat than concrete, clamshell, and

TABLE 2.

Physical measurements of cultch materials used in field and laboratory experiments.

	Clamshell	Limestone	Gravel	Concrete	Gypsum
Liquid volume of one dry liter of material (ml) .....	385.0	512.9	562.1	489.3	436.7
Weight of one dry liter of material (kg) .....	0.69	1.30	1.67	0.93	1.03
Surface area of one dry liter of material (cm <sup>2</sup> ) .....	2671	2036	2310	1434	1235
Volume (yd <sup>3</sup> ) of cultch required to cover an acre with 3 cm of material, adjusted for sinkage .....	174.8	375.7	453.4	535.2	—
Sinkage coefficient (standardized to clamshell) .....	1.0	2.5	3.0	3.2	—

TABLE 3.

Relative cost effectiveness of each cultch, based on set data from the Grand Terre field experiment and costs of materials delivered from New Orleans, Louisiana. (Costs are inflated due to a poor set and are for comparative purposes only.)

	Clamshell	Limestone	Gravel	Concrete
Average number of spat/dollar.....	0.32	0.44	0.06	0.38
Dollars/spat.....	3.15	2.26	17.45	2.64
Dollars/spat (multiplied by sinkage coefficient).....	3.15	5.63	52.35	8.40

gravel, respectively (Table 3). Values were calculated from the natural set numbers on cultches (Table 1) and material costs for delivered cultch (Table 4). If sinkage coefficients (Table 2) are taken into account, clamshell is the most cost effective being 1.8, 2.7, and 16.6 times more effective than limestone, concrete, and gravel, respectively (Table 3).

#### Laboratory Experiment

Hatchery water temperature was 29.5°C, whereas salinity was 15 ppt. The means (and ranges) of spat per liter for clamshell, limestone, concrete, and gravel were 1114 (range = 725–2179; C.V. = 0.31), 916 (range = 323–1566; C.V. = 0.42), 462 (range = 227–844; C.V. = 0.36), and 137 (range = 30–350; C.V. = 0.62), respectively (Table 5). Analysis of variance of spat per liter of cultch for the laboratory experiment shows that there was no significant difference in spatfall between clamshell and limestone. All other possible two-way comparisons were found to be significantly ( $P < 0.05$ ) different with limestone and clamshell being significantly greater in spat attracting ability than concrete and gravel.

#### Cultchless Experiments

#### Solubility Experiments

The average temperatures of the two static systems and from the two flowing systems were 20.0, 19.9, 23.8, and 20.0°C, respectively; likewise, salinity was 17, 8, 9, and 7 ppt, respectively. Gypsum dissolved at a rate of 7% a week in the static seawater systems and 25% a week in the flowing systems.

#### Hatchery Experiments

Average number of spat per cm<sup>2</sup> was 3.27 (range = 0.76–12.92; C.V. = 0.80) for gypsum and 2.73 (range = 0.07–11.57; C.V. = 0.93) for clamshell. Thus, although spat set was 1.2 times greater on gypsum than on clamshell, the analysis of variance showed that there was no significant difference between the two.

Shell height measurements of 14 day old spat averaged 0.30 mm. (Water temperature was 28.5°C and salinity was 18 ppt.)

#### DISCUSSION

Results from both field and laboratory experiments confirm reports by Chatry et al. (1986) that limestone is a biologically suitable cultch for oysters. A 1.0:1.9 ratio of clamshell to limestone (oyster spat per liter of material) in the field experiment corresponds closely with the 1.0:2.1 ratio observed by Chatry et al. (1986). Clamshell attracted more spat than roadbed, is lighter and thus is a superior cultch. Furthermore, crushed roadbed is a heavy heterogeneous material of inconsistent quality and may contain hydrocarbon and other pollutants. Gravel attracted relatively few spat in field and hatchery experiments, indicating that it is not a biologically acceptable alternative.

It is not known if the larvae are induced to settle in response to the chemical composition of the substrate, substrate texture, or chemicals associated with bacteria on the substrate. Gravel, because its chemical composition is similar to sand, may be interpreted by setting larvae as an unfavorable substrate, or its smooth surface may also be unfavorable (Ray, personal communication). Colonization by bacteria may be important in conditioning the substrate for the subsequent settlement of various invertebrates (Crisp 1967). Minimal setting of other invertebrates on gravel corresponded with minimal setting of oyster larvae. Temporal relationships of other invertebrates attaching to the other cultch materials were similar to those found by previous studies (Woods Hole 1952, Shaw 1967, Kennedy 1980).

The problem of benthic sinkage must be considered for the placement of cultch material on softer sediments. Results of the sinkage experiment corresponded well with the density of the cultches. These results indicate that clamshell, since it sinks least, would be the preferred cultch on softer water bottoms. This preference may decrease, however, as availability of clamshell decreases and cost increases. Limestone is the most cost effective cultch if benthic sinkage is not considered and thus the preferred

TABLE 4.

Purchase and transportation costs of cultch materials in May, 1990, based on purchase in New Orleans, Louisiana.

	Clamshell	Limestone	Gravel	Concrete
Dollars/yard <sup>3</sup> .....	12.75	17.40	16.50	14.30
Dollars/yard <sup>3</sup> delivered by barge in a 50 mile radius.....	14.75	19.70	18.50	16.30
Dollars/bargeload <sup>1</sup> .....	15300	21700	17160	19800
Dollars/acre (to a height of 3 cm).....	2025	2763	2620	2271
Dollars/acre (multiplied by sinkage coefficient).....	2025	6908	7860	7267

<sup>1</sup> A bargeload is about 1200 yd<sup>3</sup>.

TABLE 5.

Oyster set on clamshell, limestone, gravel, and concrete in Texas A&amp;M laboratory experiment as recorded in June 1990.

	Clamshell	Limestone	Gravel	Concrete
Number of spat/subplot .....	728.8	871.0	117.4	456.7
Total spat/total subplots.....	15,305	18,291	2,465	9,591
Average spat/l .....	1114.4	915.9	137.2	461.6
—ranges	724.5–2178.8	323.0–1565.5	29.7–350.0	227.2–843.8
—C.V.	0.311	0.416	0.617	0.358
Number of spat/cm <sup>2</sup> .....	0.42	0.45	0.06	0.31
—ranges	0.27–0.82	0.16–0.77	0.01–0.15	0.16–0.58
—C.V.	0.311	0.416	0.616	0.358

cultch for the rehabilitation of hard bottoms. Caution, in the form of small-scale, *in situ* plantings of limestone on soft-bottoms, is warranted before large-scale applications are made.

Gypsum can be used to produce cultchless oysters. The techniques described may prove useful to both mariculture and research applications. Spat became detached approximately 36 hours after setting. Spat which detach much sooner may not fully metamorphose; thus, since dissolution is related to water flow rate, water flow must be adjusted to minimize the dissolution rate of the gypsum yet maintain water quality. Favorable sets of oysters on gypsum indicate that calcium, and not necessarily calcium carbonate, may be an important component of a superior cultch material.

Limestone should prove to be an economically feasible, biologically acceptable, and environmentally benign alternative to clamshell as cultch for oysters. Crushed roadbed and gravel are not viable alternatives. Gypsum attracted setting larvae and, upon dissolution in sea water, provided cultchless spat. Raw gypsum can thus be used to produce cultchless oysters, whereas if gypsum could be stabilized it should be a suitable cultch material. Further

work needs to be conducted on the potential of raw gypsum for producing cultchless oysters and the use of stabilized gypsum as oyster cultch.

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## A POLYCLONAL ANTIBODY DEVELOPED FROM *PERKINSUS MARINUS* HYPNOSPORES FAILS TO CROSS REACT WITH OTHER LIFE STAGES OF *P. MARINUS* IN OYSTER (*CRASSOSTREA VIRGINICA*) TISSUES

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**ABSTRACT** Polyclonal antiserum was produced from *Perkinsus marinus* hypnospores harvested from oyster tissue cultivated in fluid thioglycollate medium. The specificity of the antiserum for hypnospores was tested using indirect sandwich ELISA with alkaline phosphatase-conjugated goat anti-rabbit IgG and indirect immunofluorescence. As little as 20 ng of hypnospore protein could be detected by ELISA. Immunofluorescence assays suggested that the antigenic material was a component of the spore cell wall. Cross-reactivity of the antiserum to other life stages of *P. marinus* present in oyster tissues could not be demonstrated by ELISA or immunofluorescence indicating that a substantial change in the antigenic properties of the cell wall occurs during spore formation. Hypnospore formation was also induced by placing *P. marinus*-infected oyster tissues into an anaerobic chamber rather than fluid thioglycollate. Spores were positively identified by ELISA, however little spore enlargement occurred suggesting that the triggering mechanism for spore formation is not the same as that for enlargement.

**KEY WORDS:** oyster, *Crassostrea*, *Perkinsus*, parasitism, antibody

### INTRODUCTION

The protozoan pathogen, *Perkinsus marinus*, infects over 80% of the oysters, *Crassostrea virginica*, in the Gulf of Mexico. Annual mortalities typically amount to 50% of the adult oyster population. Oysters infected with *P. marinus* often exhibit reduced growth and fecundity (Menzel and Hopkins 1955, Mackin 1962, Wilson et al. 1988, Choi et al. 1989) and changes in biochemical composition (Soniak and Koenig 1982, White et al. 1988, Wilson et al. 1988). Transmission occurs through the water (Ray 1954, Mackin 1962) or by ectoparasitic snails, *Boonea impressa* (White et al. 1987, 1989).

Ray (1952, 1966) developed a method for diagnosis of the infection using an anaerobic fluid thioglycollate medium (FTM) fortified with antibiotics. During incubation in the medium, *P. marinus* enlarges without reproduction to form hypnospores (Stein and Mackin 1957, Mackin 1962). The enlarged spores are identified microscopically by treating them with Lugol's iodine which stains the spores a dark blue or brown.

Mackin (1962) devised a semiquantitative numerical scale from 0 (uninfected) to 5 (heavily infected) for determination of the infection intensity of *P. marinus* based upon microscopic examination of the FTM-cultured oyster tissues. Most studies have used this or a slightly modified method (e.g. Mackin 1962, Sparks 1985, Crosby and Roberts 1990). Choi et al. (1989) developed a quantitative technique for determining infection intensity of *P. marinus*. The hypnospores were extracted from parasitized oyster tissues cultivated in FTM using 2 M sodium hydroxide. Oyster tissues and other parasitic organisms in the oyster tissues such as *Nematopsis* were completely dissolved in 2 M NaOH. The number of *P. marinus* cells present was then directly counted microscopically using a cell counter. Choi et al. (1989) reported that some negatives (0 on Mackin's scale) actually contain hypnospores when sufficient tissue was examined. The frequency of false neg-

atives has recently been addressed directly using incubated hemolymph and hypnospore number quantitated in a way similar to Choi et al. (1989) by Gauthier and Fisher (1990).

The potential value of immunological techniques for detecting certain marine pathogens has been established (e.g. Bower et al. 1989, Lewis 1986). Antibody to the pathogen of interest can be used to visualize or quantify infection intensity using various immunological techniques (Yentsch et al. 1988). The present report describes a procedure for producing antibodies to *P. marinus* hypnospores and its application to identification and quantification of the parasite.

### MATERIALS AND METHODS

#### Preparation of Antigen

Seven to 10 market-sized oysters were diced and transferred into 100 ml of fluid thioglycollate medium (FTM) fortified with antibiotics (Ray 1966). Hypnospores released from the oyster tissue were found in the medium after two weeks incubation. The medium containing hypnospores was filtered through cheese cloth to remove oyster tissue debris. The filtrates were then centrifuged at 1100 × g for 10 min. FTM and small oyster tissue particles found at the top of the spore pellet were removed with a Pasteur pipette connected to a vacuum pump. The pellet was resuspended in 40 ml PBS (.15 M NaCl, .003 M KCl, 0.01 M phosphate buffer, pH 7.3) and spun at the same speed and time at least 4 times. Oyster tissue particles found over the spore pellet were removed by pipette after each washing.

Percoll (Pharmacia, New Jersey) was used to separate the spores from the remaining impurities. Five ml spore preparation was added to 35 ml 80% Percoll (diluted with PBS) and centrifuged at 1200 × g for 10 min. *P. marinus* hypnospores adhering to each other formed a 'float' at the top of the centrifuge tube. The

spore layer was transferred into 35 ml 50% Percoll and centrifuged at the same speed and time. Finally, the spore layer was mixed with 35 ml 15% Percoll and spun again. The purified spores were resuspended in PBS and the Percoll residue removed by a low speed centrifugation ( $1000 \times g$  for 10 min).

Five volumes of lysis buffer consisting of 0.5% NP-40 (a non-ionic detergent), 0.15 M NaCl, and 0.01 M  $\text{KH}_2\text{PO}_4$  were added to the purified spore suspension. The cells were sonicated for 5 min in an ice-filled bucket using an Ultrasonic Cell Disrupter to extract the cell protein. Including the detergent in the lysis buffer greatly improved the efficiency of protein extraction compared to simply rupturing the cells with the sonicator. The protein extract was dialyzed against PBS using a 6000-8000 molecular weight dialysis membrane for 3 days with 2 changes (each of 3000 ml) of PBS per day to remove the NP-40. After dialysis, total protein in the extract was estimated using the BCA Protein Assay (Pierce, Illinois).

#### Production of Antisera

An albino New Zealand rabbit initially received a 1 ml subcutaneous injection of hypnosporin protein extract (1 mg protein  $\text{ml}^{-1}$ ) prepared as just described mixed with an equal volume of Freund's complete adjuvant. After the initial injection, the rabbit received 0.5 ml (500  $\mu\text{g}$ ) *P. marinus* hypnosporin protein mixed with 0.5 ml Freund's incomplete adjuvant on a weekly basis over 5 weeks. After the fourth week, 10 ml of blood was drawn from the rabbit and the immune response assessed using a precipitin ring test and a passive hemagglutination test with antigen-coated sheep red blood cells (Garvey et al. 1977). Forty ml of blood was withdrawn 14 days after the last injection to obtain the antiserum for the study. The antiserum was absorbed with acetone-dried uninfected oyster tissue (1.0 g) and acetone-dried purified oyster egg powder to remove the nonspecific antibody present in the serum. Uninfected oyster tissue was obtained from the Mississippi River, Tiger Pass site sampled by NOAA's Status and Trends "Mussel Watch" Program (Powell et al. in press). This site has been completely free of *P. marinus* for at least 3 consecutive years. Rabbit anti-*P. marinus* immunoglobulin G (IgG) was precipitated from the serum using half-saturated ammonium sulfate precipitation according to Garvey et al. (1977). The extracted IgG was resuspended into 40 ml of PBS and excess ammonium sulfate removed by dialysis in PBS. The IgG was stored at  $-60^\circ\text{C}$  until used.

#### Oyster Sample Preparation

Market-sized oysters were collected from Galveston Bay during July–November 1988. After removing excess water, the wet weight of each oyster was recorded. A piece of mantle tissue was excised and added to 10 ml FTM fortified with antibiotics. After two weeks of incubation, *P. marinus* infection intensity was rated on the 0 (uninfected) to 5 (heavily infected) point scale of Mackin (1962) according to Craig et al. (1989). Oyster hemolymph was also obtained from 5 oysters (5 ml total) and a 500  $\mu\text{l}$  aliquot analyzed for the presence of *P. marinus* using the FTM technique. The hemolymph *P. marinus* were ruptured using an Ultrasonic Cell Disrupter to release *P. marinus* protein. Oyster tissue was first homogenized into PBS using a glass-syringe tissue grinder and then sonicated to extract *P. marinus* protein. A 1 ml aliquot of each sample was centrifuged to remove the tissue debris and the supernatant stored frozen for further analysis.

#### Enzyme Linked Immunosorbent Assay (ELISA)

Polystyrene 96-well ELISA microtitre plates (Becton Dickinson Labware, New Jersey) were used. Fifty  $\mu\text{l}$  of oyster tissue or serum preparation were coated on the well and incubated at  $4^\circ\text{C}$  overnight or at  $37^\circ\text{C}$  for 4 to 6 hr. Each plate included, as positive controls, various dilutions of hypnosporin protein extract and, as negative controls, homogenates of uninfected oyster tissue preparation. After incubation, the plate was washed twice with PBS containing 0.05% Tween-20 (PBS T-20) and 300  $\mu\text{l}$  of 1% w/v bovine serum albumin were added to block nonspecific-binding sites. The plate was incubated for 1 hr at room temperature and washed twice with PBS T-20. Rabbit anti-*P. marinus* hypnosporin IgG (5 to 10  $\mu\text{g ml}^{-1}$ ) was added in 50- $\mu\text{l}$  aliquots to each well; the plate was incubated for 1 hr at room temperature and washed three times with PBS T-20. Diluted (1/200) goat anti-rabbit serum IgG alkaline phosphatase conjugate (Zymed Laboratories, California) was added in 50- $\mu\text{l}$  aliquots to each well, the plate incubated again for 1 hr at room temperature and washed 5 times with PBS T-20 after incubation. Fifty  $\mu\text{l}$  of substrate (*p*-nitrophenylphosphate dissolved in diethanolamine buffer) (KPL Inc., Maryland) was added. After 30 min to 1 hr incubation at room temperature, optical density of the colored reaction products was measured immediately at 405 nm with an ELISA reader.

#### Immunofluorescence Assay

FTM-cultured and uncultured oyster tissues and *Nematopsis*-infected oyster tissue were quick frozen in liquid nitrogen-isopentane. Sections were cut at 2  $\mu\text{m}$  on a cryostat, washed twice for 10 min in PBS T-20 and incubated in a humid container with a 1/500 dilution of rabbit anti-*P. marinus* IgG for 1 hr. The sections were washed twice with PBS T-20 and incubated in a humid container with a 1/50 dilution of goat anti-rabbit fluorescein isothiocyanate-conjugated (FITC) IgG for 1 hr. After twice washing the sections with PBS T-20, they were examined under an ultraviolet light microscope.

Examination of purified hypnosporins involved placing 0.5 ml pellet volume of purified *P. marinus* spores into 5 ml of rabbit anti-*P. marinus* IgG (1 mg  $\text{ml}^{-1}$ ) and shaking the suspension overnight at room temperature. The spores were centrifuged at  $1100 \times g$  for 10 min to remove the unbound IgG and washed three times with 10 ml PBS T-20, by centrifugation ( $1100 \times g$  for 10 min). Five ml of 1/10 diluted goat anti-rabbit FITC IgG was added. The spores were incubated for 2 hr at room temperature and washed 3 times with PBS T-20. The stained hypnosporins were mounted on microscope slides and examined under ultraviolet light.

## RESULTS

#### Immunofluorescence Assay

Plate 1 shows *P. marinus* hypnosporins stained with FITC goat anti-rabbit IgG. Fluorescence was observed on the surface of hypnosporins suggesting that the preparation included antibodies developed against proteins located on the surface of the hypnosporins. Fluorescence was also observed on the surface of hypnosporin cell walls from the cryostat sections of FTM-incubated tissue. Nonspecific staining of oyster tissue free of hypnosporins, unincubated oyster tissue, or other parasitic organisms, particularly *Nematopsis*, was not observed.

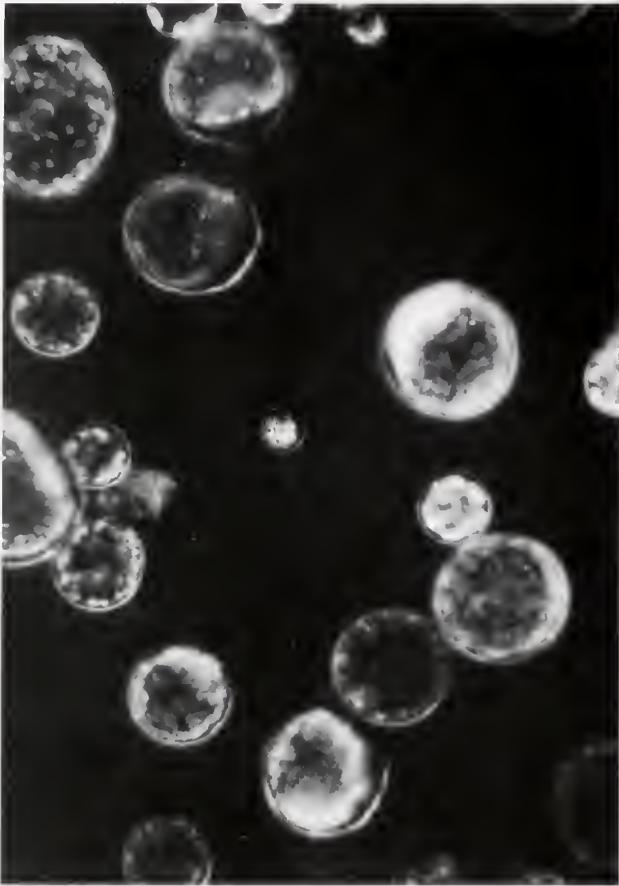


Plate 1. *P. marinus* hyphospores stained with FITC goat anti-rabbit IgG.

#### ELISA on Hyphospores and Live Oyster Tissues

Indirect sandwich ELISA was selected as a quantitative assay for hyphospore antigen. Figure 1 shows a typical ELISA titration curve for rabbit anti-*P. marinus* hyphospores. The curve indicates that the antibody used in ELISA detects as little as  $20 \text{ ng ml}^{-1}$  *P. marinus* hyphospore protein. During ELISA, the antiserum exhibited a slight cross-reactivity to oyster tissue. After absorbing the

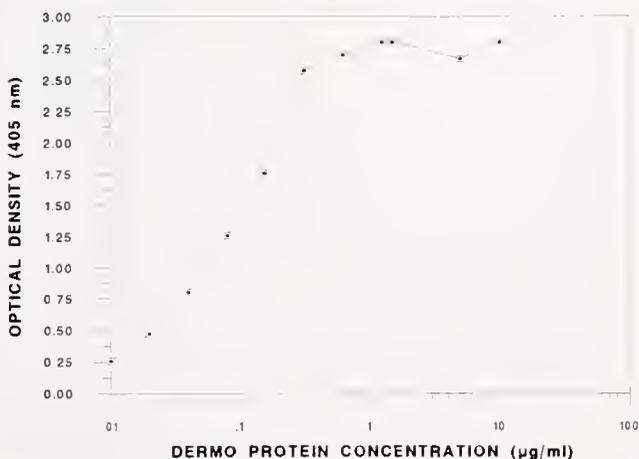


Figure 1. A typical ELISA calibration curve for rabbit anti-*P. marinus* IgG.

primary serum with acetone-dried oyster tissue free of *P. marinus*, no further cross-reactivity was observed. Twofold serial dilutions up to 1/2048 were made from a 1 ml oyster tissue homogenate to test the cross-reactivity of the antiserum to other life stages of *P. marinus* present in live oyster tissues. The optical densities (O.D.) of each sample measured from ELISA were then compared with the O.D. of positive controls from protein extracted from *P. marinus* hyphospores. The ratio between the O.D. of the oyster samples and the O.D. of the positive controls observed at the midpoint of the titration curve was calculated to determine the cross-reactivity. Any oyster samples with the ratio O.D. of sample to O.D. of the positive control greater than 1 were considered to exhibit positive cross-reactivity with rabbit anti-*P. marinus* hyphospore IgG.

Twenty *P. marinus*-infected oysters and one oyster hemolymph sample were tested using the ELISA assay. Infection levels, estimated from Choi et al.'s (1989) calibration of Ray's (1966) assay, ranged from  $1.5 \times 10^4$  to  $1.1 \times 10^7$  cells g wet wt oyster<sup>-1</sup>. No significant variation of the ratio O.D. sample to O.D. positive control occurred among the oyster samples. Few O.D.s measured on the oyster samples from ELISA exceeded the O.D. measured on  $80 \text{ ng ml}^{-1}$  hyphospore protein. None exhibited a strong positive reaction typical of FTM-incubated tissue ( $>1 \mu\text{g ml}^{-1}$ ). Moreover, the O.D. measured on uninfected oyster tissues, used as negative controls, did not differ from the O.D. measured on moderately-heavily or heavily infected oyster tissues in the ELISA test.

#### DISCUSSION

*Perkinsus marinus* has several life stages in the host oyster (Mackin and Boswell 1956, Perkins 1969). These include immature thalli, mature unicellular thalli (trophozoites—Perkins 1987), and presporangia. When liberated into sea water, presporangia develop a resistant cell wall and enlarge to become hyphospores. Under aerobic conditions in seawater, hyphospores differentiate into sporangia and produce motile zoospores (aplanospores in Mackin and Boswell 1956) within the hyphospore cell wall. Zoospores are then discharged from the hyphospores and undergo a free living stage in sea water (Perkins and Menzel 1966, Perkins 1969). Hyphospores are also developed when infected oyster tissues are placed into FTM (Ray 1952) or sterilized oyster serum (Mackin 1962). However, hyphospores characterized by a thickened wall, increased size, or dark blue or brown-staining when treated with Lugol's iodine are rarely seen in live oyster tissues (Mackin

TABLE 1.

ELISA titration curve data for rabbit anti *P. marinus* IgG.

<i>P. marinus</i> Protein ( $\mu\text{g/ml}$ )	Optical Density (405 nm)
10	2.800
5	2.670
2.5	2.900
1.25	2.800
0.625	2.700
0.313	2.580
0.156	1.762
0.08	1.262
0.04	0.808
0.02	0.476
0.01	0.259

1962). What causes hypnospor formation or the role of hypnosporos in the life cycle remains unclear. Mackin (1962) postulated that hypnosporos may serve as overwintering spores.

We induced hypnospor formation from infected oyster tissues by placing them in a test tube partly filled with PBS and cultured in a carbon dioxide filled anaerobic chamber. After a 72 hr incubation period, all cultured oyster tissues produced spores. Unlike FTM culture however, little enlargement occurred. Spores were 20 to 40  $\mu\text{m}$  in diameter, only about 2 to 4 times the size of the thalli usually found in infected tissue. These were identified microscopically as hypnosporos by staining with Lugol's iodine. An ELISA test using rabbit anti-hypnospor as primary antibody showed a strong positive reaction to these tissues confirming that the spores developed in the anaerobic chambers are similar in cell wall protein composition to hypnosporos formed in FTM culture.

Stein and Mackin (1957) also observed little enlargement in sterile seawater, even after 9 days incubation. Accordingly, the development of characteristic hypnospor proteins and concurrent enlargement are probably distinct phenomena. The anaerobic conditions present in decomposing oyster tissue appear sufficient to trigger the development of a protein complement typical of *P. marinus* hypnosporos but are not sufficient to trigger the degree of enlargement typical of cells in FTM. The same phenomenon, hypnospor production with little enlargement, has occasionally been noted in normal thioglycollate culture. At least one proposed life cycle includes parallel spore stages with varying degrees of enlargement (Mackin and Boswell 1956).

Rabbit antiserum from hypnosporos failed to cross-react with other stages of *P. marinus* present in oyster tissue. Immunofluorescence tests conducted on hypnosporos extracted from FTM culture indicated that the antigens recognized by rabbit antiserum are present on the surface of hypnospor cell walls. Immunofluorescence was not observed in uncultured, infected oyster tissue. Homogenates of hypnosporos reacted positively in ELISA. Homoge-

nates of infected oyster tissue did not, without prior anaerobic incubation or culturing in FTM. At least some of the proteins present on the surface of hypnosporos as seen on the immunofluorescence micrograph are unique: they are present in hypnosporos but not in detectable quantities in life stages of *P. marinus* normally found in oyster tissue. The data suggest that spore formation results in a substantial change in cell wall antigenic complement.

Furthermore, the substantial cross-reactivity of the initial spore antibody preparation with uninfected oyster tissue suggests, although does not prove, that the normal life stages of *P. marinus* in oyster tissue contain a substantial oyster-like antigenic component. The spore preparation used for injection into the rabbits was free of oyster tissue debris. The oyster antigenic complement could have come from one of two sources. (1) Perkins (1969) observed what was interpreted to be oyster cell membranes embedded in the walls of *P. marinus* found in oyster tissue. The role of embedded cell membranes from the host oyster in the *P. marinus* cell wall is unknown; their presence would explain the cross-reactivity we observed, however, and might explain the poor recognition of *P. marinus* by oyster hemocytes considered by Cheng (1987) (but see Mackin 1951). Certainly our data conform with Perkin's (1969) interpretation. (2) Oyster proteins may adhere to the cell surface; other protozoa use this protective mechanism to mask foreign protein. In contrast, Chu (1988) observed an immune response to zoosporos, suggesting that all life stages not normally found in oyster host tissue might be characterized by cell wall protein components substantially different from those found in the parasitic stages.

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## EFFECTS OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*: I. SUSCEPTIBILITY OF NATIVE AND MSX-RESISTANT STOCKS

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**ABSTRACT** A selective breeding program was implemented to attempt to decrease the disease susceptibility of the eastern oyster, *Crassostrea virginica*, to *Perkinsus marinus*. Six oyster strains were spawned and the progeny exposed to *Haplosporidium nelsoni* (MSX) and *P. marinus* in the lower Chesapeake Bay. Three strains, a Delaware Bay MSX-resistant strain, a Delaware Bay native strain, and a Mobjack Bay native strain (lower Chesapeake Bay) were exposed for three years (1988-90); three other strains, a separate Delaware Bay MSX-resistant strain, a lower James River native strain (lower Chesapeake Bay) and a susceptible control strain, were exposed for two years (1989-90). During the study period, *P. marinus* abundance was high and increased each year; *H. nelsoni* abundance was low and decreased each year. Both strains of MSX-resistant oysters developed by Rutgers University were highly susceptible to *P. marinus*. Cumulative mortality at the end of the study was 99% for both strains and growth virtually stopped after acquisition of *P. marinus*. Mean shell height did not reach market size in either MSX-resistant strain. All native strains (Delaware Bay, Mobjack Bay and James River) had about 80% cumulative mortality, mainly from *P. marinus*, but the Mobjack Bay strain also experienced moderate mortality from *H. nelsoni*. However, these strains continued growing and survivors reached market size during the study period. The MSX-resistant strains offer little immediate benefit in a selective breeding program for the Chesapeake Bay oyster industry because of their high susceptibility to *P. marinus* and poor growth; however, they may be valuable, especially in crosses with native strains, during periods of *H. nelsoni* resurgence and *P. marinus* decline. The three native strains performed better than the resistant strains and will be utilized, both as direct lines and as intraspecific hybrids, in a continuing selective breeding program to decrease the disease susceptibility of *C. virginica* stocks.

**KEY WORDS:** oysters, disease, Chesapeake Bay, *Haplosporidium*, growth, mortality

### INTRODUCTION

The oyster industry in Virginia has been in serious decline since 1960. Between 1932 and 1960 oyster landings in Virginia actually increased, reaching a peak of 4.0 million bushels in 1959 (Hargis and Haven 1988). This increase was primarily the result of a dramatic increase in landings from the private sector of the industry; landings from public beds continued to decline gradually during the period and accounted for only about 20% of the total harvest by the late 1950s. Beginning in 1960, the well documented MSX epizootic, caused by the protozoan *Haplosporidium nelsoni* (Haskin, Stauber and Mackin), caused large scale mortality and eventually resulted in abandonment of traditional leased beds in high salinity portions of the lower Chesapeake Bay (Haskin and Andrews 1988). As a result, oyster landings from the private sector declined precipitously during the 1960s and early 1970s and by 1974 landings from private and public beds were approximately equal (Hargis and Haven 1988). During the 1970s and early 1980s, landings were relatively stable, although very low compared with pre-1960 levels. Unfortunately, successive drought years from 1985 through 1988 caused a resurgence of *H. nelsoni* in Chesapeake Bay (Burreson and Andrews 1988, Haskin and Andrews 1988) and also an unprecedented intensification and spread of another protozoan pathogen, *Perkinsus marinus* (Mackin, Owen and Collier) to all oyster beds in Virginia (Andrews 1988, Burreson and Andrews 1988). High oyster mortality from combined effects of the two diseases during this period resulted in successive record low landings each year from 1988 through 1990 (Virginia Marine Resources Commission, landings records).

Although many factors, including overharvesting, are contributing to the continuing decline of the Virginia oyster industry

(Hargis and Haven 1988), it is clear that disease-induced mortality directly, or reduced planting in the private sector because of the fear of high losses, were primarily responsible for the rapid decline in landings during the 1960s and again in the 1980s. Therefore, it seems unlikely that the industry can be rehabilitated rapidly unless oysters can be developed that are less susceptible to disease. One approach to developing resistant oysters is through a selective breeding program in which surviving oysters from disease endemic areas are selected and bred over successive generations in an attempt to decrease disease-induced mortality. This approach has been used successfully to increase the survival of oysters exposed to *H. nelsoni* (MSX) (Andrews 1968, Haskin and Ford 1979, Ford and Haskin 1987, Ford 1988, Haskin and Andrews 1988). Between 1960 and 1985, *H. nelsoni* was responsible for most of the disease-induced mortality in Chesapeake Bay oysters. However, since 1985, *P. marinus* has gradually replaced *H. nelsoni* as the most important oyster pathogen in Chesapeake Bay (Burreson and Andrews 1988, Andrews 1988) and little effort has been devoted to developing oysters with decreased susceptibility to this pathogen. The purpose of this paper is to compare the susceptibility to *P. marinus* of oysters bred for decreased susceptibility to *H. nelsoni* and of surviving oysters from various disease endemic areas in the lower Chesapeake Bay. Results will be used to choose broodstock for a continuing selective breeding program to increase survival of oysters exposed to both local diseases.

### METHODS

Hatchery-reared broods from six stocks of oysters were utilized in this study:

1. MSX-selected A: Delaware Bay native strain selected through six generations in Delaware Bay for resistance to *H. nelsoni*. These oysters demonstrated significantly greater

survival than unselected control oysters (Ford and Haskin 1987) when exposed to *H. nelsoni*. Broodstock (Rutgers University BXF) was provided by Drs. H. Haskin and S. Ford.

2. Delaware Bay natives: native oysters from the lower seed area in Delaware Bay provided by Drs. H. Haskin and S. Ford, Rutgers University.
3. Mobjack Bay natives: oysters from a population on Pultz Bar in Mobjack Bay, VA that has suffered annual exposure to both *H. nelsoni* and *P. marinus* since 1959.
4. MSX-selected B: upper James River native strain selected through five generations in Delaware Bay for resistance to *H. nelsoni*. These oysters demonstrated significantly greater survival than unselected control oysters (Ford and Haskin 1987) when exposed only to *H. nelsoni* in Delaware Bay. Broodstock (Rutgers University AVA2A) was provided by Drs. H. Haskin and S. Ford.
5. Lower James River natives: oysters from a population on Nansmond Ridge in the lower James River that has suffered annual exposure to both *H. nelsoni* and *P. marinus* since 1960 and severe exposure to *P. marinus* since 1985.
6. Susceptible controls: oysters from Horsehead Rock, until 1988 a low salinity sanctuary from disease in the upper James River. Oysters from this location have historically exhibited high disease susceptibility to both *H. nelsoni* and *P. marinus* and are used routinely in the oyster disease monitoring programs conducted by the Virginia Institute of Marine Science (VIMS) and Rutgers University.

Broodstock from all stocks were conditioned at 22°C in the VIMS hatchery. Spawning was induced by raising the water temperature to 30°C; all spawnings used at least 10 individuals. If elevated temperature did not induce spawning, a male from the broodstock group was stripped and sperm added near the incumbent region of each oyster by pipet. When oysters spawned, they were identified as to sex and placed in separate containers to collect sperm and eggs; eggs from the spawning trough were collected on a 20 µm sieve and added to the egg container. Eggs were fertilized by the addition of sperm. Larvae were reared in 400 gallon conical tanks, set on minicultch and hardened in upwellers. When spat were large enough to be transferred from upwellers to flumes they were placed in small 6.0 mm mesh bags and held in Nestier trays in seawater flumes until deployed for field challenge. Because of oyster and hatchery availability, not all stocks were spawned at the same time. Stocks 1–3 (above) were spawned in November, 1987 and oysters were approximately 6 mo old when placed in the York River for exposure to diseases on 1 May 1988. Stocks 1–3 were monitored in the river until September 1990. Stocks 4–6 (above) were spawned in April, 1988 and were approximately 1 yr old when disease challenge was initiated on 1 May 1989. Stocks 4–6 were monitored in the river until December 1990. Spat from all strains were singled ( $n \geq 1000$ ) and placed into labeled nylon 6.0 mm mesh bags that were held in  $0.6 \times 1.2$  m legged oyster trays. The tray frames were covered with 2.5 cm plastic mesh to exclude large predators. Trays were suspended from a pier at VIMS in the lower York River, an endemic area for both *H. nelsoni* and *P. marinus*.

For comparison with experimental strains, susceptible oysters (2 to 3 inches shell height) from the routine VIMS oyster disease monitoring trays were used to assess the annual prevalence and intensity of both *H. nelsoni* and *P. marinus*. These trays were

identical in construction to those mentioned above and contained 500 oysters collected in late April each year from either Horsehead Rock or Deepwater Shoal in the upper James River. Oysters from these beds have only rarely been exposed to *H. nelsoni* and *P. marinus* and are highly susceptible to both pathogens. The trays are typically deployed on 1 May each year and removed on 1 December and provide a long term record of annual disease severity.

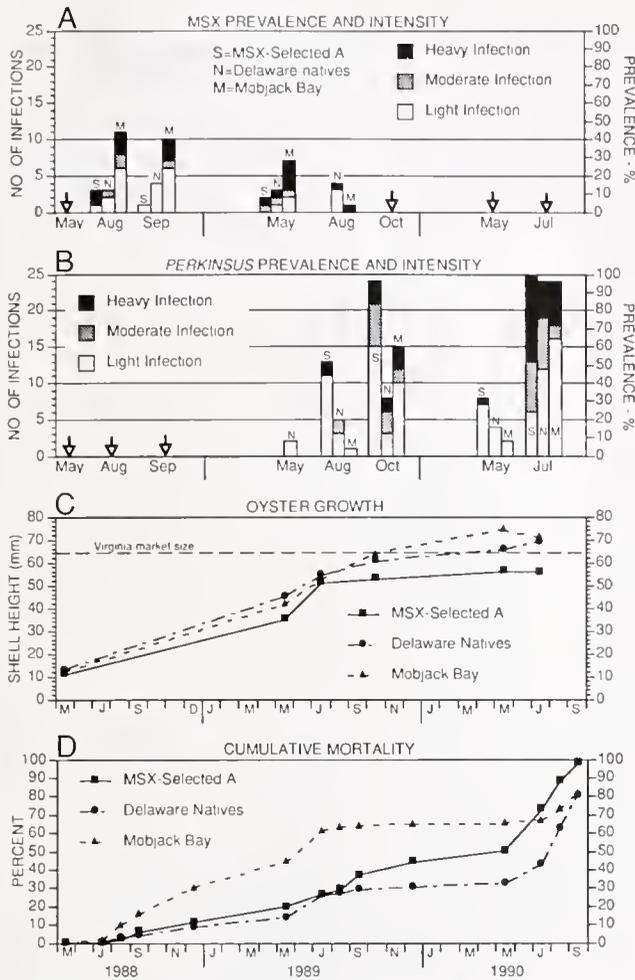
Live and dead oysters in trays were counted every two weeks from 15 May until 1 December. Weekly counts were usually made during periods of high mortality. Samples of 25 oysters for disease diagnosis were removed periodically from each tray; samples were usually taken in May, July or August and September or October. *Perkinsus marinus* was diagnosed in all samples by thioglycollate culture (Ray 1952) of mantle, gill and rectal tissue; *H. nelsoni* was diagnosed by routine paraffin histology of oysters preserved in Davidson's AFA. A subsample of 100 oysters from each group was measured for total shell height in May of each year and also in July and late September of the second and third years.

Differences in shell height among the various oyster strains were analyzed by one-way ANOVA with subsequent Scheffé multiple comparison tests. Differences in parasite prevalence, intensity and oyster mortality between groups were analyzed by chi-square contingency tables with continuity correction. For prevalence comparisons, the contingency table columns were numbers of infected and uninfected oysters; for intensity comparisons one column was the sum of the number of heavy and moderate infections and the other column was the number of light infections. For mortality comparisons the columns were the number of live and dead oysters to that date. Table rows were the various strains of oysters. All statistical tests were run on an Apple Macintosh II using Statview II.

## RESULTS

Prevalence and intensity of *H. nelsoni* (MSX) and *P. marinus*, oyster growth, and cumulative oyster mortality for experimental oyster stocks 1–3 are shown in Figure 1. There was no difference in growth among the three stocks during the first year (Fig. 1C). However, cumulative mortality was significantly greater ( $P < 0.01$ ) in the Mobjack Bay stock than either the MSX-selected A or the Delaware Bay native stock from December 1988 through May 1990. The higher mortality in the Mobjack Bay stock appears to be the result of significantly higher ( $P < 0.01$ ) MSX prevalence in this stock (Fig. 1A) in August and September 1988. No *P. marinus* was observed in any of these three stocks during 1988 (Fig. 1B) although prevalence in market size control oysters reached 68% in August (Table 1) indicating that *P. marinus* was abundant during the period. Prevalence of MSX was also greater in the market size control oysters (Table 1) than in any of the experimental stocks 1–3 during 1988 and 1989 (Fig. 1A).

During 1989, the second year of exposure, experimental stocks 1–3 all became infected with *P. marinus* (Fig. 1B). Prevalence of *P. marinus* was significantly greater ( $P < 0.01$ ) in the MSX-selected A stock than in the other two stocks during August and October 1989. The prevalence of MSX gradually declined during this period and MSX was absent in October 1989 samples of experimental stocks (Fig. 1A) even though prevalence and intensity of MSX was high in market size control oysters (Table 1). The high prevalence of *P. marinus* in the MSX-selected A stock re-



**Figure 1.** Results from oyster stocks deployed in May 1988. In A and B total bar height is the number of infected oysters (left axis) and prevalence (right axis) in a sample of 25 oysters. Arrows indicate samples examined, but no infections found. Oyster stock designations listed in A also apply to B. Time line shown in D applies to all figures.

sulted in increased cumulative mortality (Fig. 1D) and greatly decreased growth (Fig. 1C, Table 2). Although the Mobjack Bay native stock had higher prevalence of *P. marinus* than the Delaware Bay native stock (Fig. 1B), the Mobjack Bay stock grew faster (Fig. 1C, Table 2) than the Delaware Bay native stock between July 1989 and May 1990. In May 1990 shell heights (Fig. 1C) and cumulative mortalities (Fig. 1D) were significantly different ( $P < 0.01$ ) between all experimental stocks 1–3. In July 1990 all experimental stocks had high prevalence of *P. marinus*, but the number of heavy and moderate infections was significantly greater ( $P < 0.01$ ) in the MSX-selected A stock (Fig. 1B) than in the other two stocks. The high prevalence and intensity of *P. marinus* resulted in greatly increased mortality in all three stocks during summer of 1990, but especially in the MSX-selected A and the Delaware Bay native stock (Fig. 1D). When the experiment was terminated in September 1990, the cumulative mortality in the MSX-selected A stock was significantly greater ( $P < 0.01$ ) than in the other two stocks. Mean shell height decreased during summer 1990 in the Mobjack Bay stock and the MSX-selected A stock (Fig. 1C, Table 2), apparently as a result of selective mortality of

large oysters. Mean shell height for the MSX-selected A stock never reached market size (Fig. 1C). No MSX was observed in the experimental stocks during 1990 and only one infection was found in the market size control oysters (Table 1).

Prevalence and intensity of *H. nelsoni* (MSX) and *P. marinus*, oyster growth and cumulative oyster mortality for experimental stocks 4–6 are shown in Figure 2. These three stocks were approximately one year old when placed in the York River and, thus, were larger than stocks 1–3 when first exposed to disease challenge (Figs. 1C, 2C; Table 2). The lower James River stock and the susceptible control stock apparently acquired MSX in the holding flume because both stocks had a low prevalence of MSX when field challenge was initiated (Fig. 2A). The lower James River stock was not infected with MSX in subsequent samples, but prevalence remained low in the susceptible control stock. No MSX was found in the MSX-selected B stock during 1989 and only 2 infected oysters were found during May 1990, although both had heavy infections (Fig. 2A). The MSX infections observed in experimental stocks during May 1990 were probably acquired during fall 1989 because no MSX infections were found during May or July 1990 in market size control oysters (Table 1) or during July or September in the experimental stocks, suggesting that typical early summer MSX infections did not occur during 1990. All three stocks acquired *P. marinus* by August 1989, but both prevalence and intensity were significantly lower ( $P < 0.05$ ) in the lower James River stock than in the other two stocks (Fig. 2B). The MSX-selected B stock seemed to be most affected by the *P. marinus* infections; shell height of the MSX-selected B stock was significantly lower ( $P < 0.05$ ) than the other two stocks in both July and October 1989 (Fig. 2C) and cumulative mortality of the MSX-selected B stock was significantly greater ( $P < 0.05$ ) by December 1989 (Fig. 2D). During 1990, both prevalence and intensity of *P. marinus* were high in July and September with no significant differences among the various stocks (Fig. 2B). Cumulative mortality of the MSX-selected B stock was significantly greater ( $P < 0.01$ ) than the other two stocks at each sampling date during 1990 (Fig. 2D). By December 1990 the cumulative mortality of the lower James River stock was also significantly lower ( $P < 0.01$ ) than the susceptible control stock. Mean shell height of the MSX-selected B stock did not reach market size and was significantly lower ( $P < 0.05$ ) than the other two stocks in July 1990 (Fig. 2C, Table 2). There were insufficient oysters remaining in September 1990 for disease diagnosis or growth measurements in the MSX-selected B stock.

**DISCUSSION**

Interpretation of the results was confounded by decreasing prevalence of *H. nelsoni* and increasing prevalence of *P. marinus* over the three year study period based on prevalence of both pathogens in the large control oysters. In addition, the six oyster stocks assessed were spawned and exposed at two different times and, thus, were two different ages at the initiation of disease challenge. Nevertheless, some important conclusions can be reached about the value of using each of these stocks in a continuing selective breeding program to decrease the disease susceptibility of *C. virginica*.

**Susceptibility to *H. nelsoni***

The two stocks selected for decreased susceptibility to *H. nelsoni* in Delaware Bay (MSX-selected A and B stocks) and the

TABLE 1.

Prevalence and intensity of *H. nelsoni* (MSX) and *P. marinus* in large susceptible control oysters imported from the upper James River in May each year to the lower York River, an endemic area for both parasites.

Date	MSX Infected/Examined	Percent Infected	H-M-L*	<i>P. marinus</i> Infected/Examined	Percent Infected	H-M-L*
1988						
01 May	0/25	0		0/25	0	
19 Jul	11/25	44	4-0-7	No sample		
17 Aug	18/25	72	1-3-14	17/25	68	3-5-9
21 Sep	12/25	48	3-0-9	14/25	56	3-3-8
1989						
01 May	0/25	0		0/25	0	
05 Jul	5/25	20	0-1-4	1/25	4	0-0-1
02 Aug	8/25	32	2-0-6	5/25	20	0-1-4
07 Sep	13/25	52	1-2-10	23/25	96	2-4-17
03 Oct	14/25	56	3-3-8	25/25	100	8-9-8
1990						
01 May	0/25	0		0/25	0	
16 Jul	0/25	0		12/25	48	3-4-5
28 Aug	1/25	4	0-0-1	25/25	100	11-9-5
26 Sep	0/30	0		28/28	100	10-15-3

\* H = number of heavy infections, M = moderate infections, L = light infections.

Delaware Bay native stock, demonstrated reduced susceptibility to *H. nelsoni* compared to the other stocks, as expected based on results of previous studies (Ford and Haskin 1987, Haskin and Andrews 1988). MSX-selected A and Delaware Bay native stocks both had significantly lower prevalence of *H. nelsoni* than the Mobjack Bay native stock during 1988, a year with high *H. nelsoni* challenge on the basis of prevalence and intensity in market size susceptible controls. No *H. nelsoni* infections were found in the MSX-selected B stock during 1989 even though the lower James River stock and the susceptible control stock were infected. The lower James River stock was not infected with MSX after May, 1989, but the susceptible control stock remained infected, at low prevalence, through May 1990. The rapid loss of infection in the lower James River stock was probably the result of mortality of heavily infected oysters; the absence of MSX infection in subsequent months may indicate decreased susceptibility in that stock because of the long history of exposure to MSX in the lower James River. Only a moderate natural *H. nelsoni* challenge occurred during 1989 and only a very low challenge occurred during 1990; thus, data on susceptibility of all six stocks to *H. nelsoni*, but especially the three stocks exposed in 1989, should be interpreted with caution.

The lower prevalence of *H. nelsoni* in the Mobjack Bay stock than in the large susceptible control oysters is probably a result of decreased susceptibility of the Mobjack Bay stock because of its history of exposure to *H. nelsoni*, although it is clearly more susceptible than the Delaware Bay native stock. An alternative explanation is that the lower prevalence of *H. nelsoni* in the Mobjack Bay stock compared with the large control oysters is the result of the smaller size of the Mobjack Bay stock oysters. Assuming that infective stages of *H. nelsoni* are water-borne, small oysters that filter less water than large oysters would acquire fewer infections and would have lower prevalence of the parasite; however, based on *H. nelsoni* infections in stocks 1-3 during 1988, it is clear that small oysters in the range of 12 to 30 mm shell height can acquire *H. nelsoni* infections. In contrast, as discussed in the

next section, oysters of that size apparently do not acquire *P. marinus*.

#### Susceptibility to *P. marinus*

All stocks of oysters examined during this study had high susceptibility to *P. marinus*, but the two stocks selected for resistance to *H. nelsoni* generally demonstrated higher *P. marinus* prevalence and intensity than the other four stocks. It is interesting that *P. marinus* prevalence and intensity were no worse in the Delaware Bay native stock than in the Mobjack Bay stock even though Mobjack Bay is an endemic area for *P. marinus* and this pathogen has only rarely been present in Delaware Bay. The lower James River stock, also from an endemic area for *P. marinus*, had lower prevalence and intensity of the parasite than other stocks during 1989, but there was no difference between stocks in 1990. These results may suggest that exposure to *P. marinus* over many generations in the James River has decreased the susceptibility to initial challenge, but not to continued disease pressure. Alternatively, the contribution to the gene pool from the unselected, disease susceptible upper James River oysters is assumed to be large and may account for the apparent low resistance in disease endemic areas.

The high susceptibility of the MSX-selected stocks to *P. marinus* suggests that the documented (Ford and Haskin 1987) decreased susceptibility to *H. nelsoni* is not a generalized response to pathogens, but is specific for *H. nelsoni*, or at least the mechanism is not effective against exposure to high levels of *P. marinus*. Not only are MSX-resistant oysters highly susceptible to *P. marinus*, but they appear to be more susceptible than less highly selected oysters, as if the selection process had actually increased the susceptibility to *P. marinus*. These results contradict the suggestion by Valiulis (1973) that there is a common survival mechanism against both pathogens; however, as discussed by Ford (1988), the evidence compiled by Valiulis was less than compelling. Ford (1988) suggested that decreased susceptibility to *H. nelsoni* in

TABLE 2.

Shell height measurements (mm) for the strains of *C. virginica* held in the lower York River at Gloucester Point, VA. N = 100 for each sample except where noted.

Oyster Strain	Date	Shell Height Mean ± S.D.	Range
MSX-Selected A	5-02-88	10.9 ± 2.1	6-19
	5-02-89	34.8 ± 8.2	18-60
	7-05-89	51.0 ± 10.7	30-75
	10-02-89	52.7 ± 9.9	29-79
	5-01-90	56.1 ± 8.7	36-77
	7-23-90	55.9 ± 9.8	31-81
Delaware Bay natives	5-02-88	11.5 ± 3.0	5-21
	5-02-89	44.8 ± 13.1	18-75
	7-05-89	54.5 ± 12.2	30-90
	10-02-89	60.6 ± 13.0	25-93
	5-01-90	65.4 ± 9.5	45-89
	7-23-90	69.2 ± 10.2	48-97
Mobjack Bay natives	5-02-88	11.1 ± 2.9	5-22
	5-02-89	41.6 ± 12.2	15-81
	7-05-89	52.5 ± 11.3	27-78
	10-02-89	63.5 ± 10.9	42-92
	5-01-90	74.5 ± 8.4	55-98
	7-23-90	71.3 ± 10.5	38-93
MSX-Selected B	5-02-89	24.1 ± 4.8	15-35
	7-05-89	35.5 ± 9.7	18-63
	10-02-89	43.7 ± 11.3	21-69
	5-01-90	50.1 ± 11.4	29-80
	7-23-90	54.7 ± 10.3	34-82
	10-08-90	No sample	
Lower James River natives	5-02-89	24.3 ± 4.3	17-35
	7-05-89	42.4 ± 8.8	24-65
	10-02-89	51.3 ± 8.2	30-68
	5-01-90	55.2 ± 10.1	30-77
	7-23-90	63.2 ± 7.5	43-78
	10-08-90	64.0 ± 7.1	47-84
Susceptible controls	5-02-89	17.9 ± 4.8	10-31
	7-05-89	42.4 ± 7.6	22-65
	10-02-89	52.1 ± 8.8	30-76
	5-01-90	59.8 ± 9.0	38-90
	7-23-90	61.5 ± 7.9	47-83
	10-08-90	63.0 ± 6.7	43-82

(N = 89)

oysters is the result of a complex of factors including cellular and humoral host responses and physiological and metabolic conditions in the host that provide unsuitable environments for parasite development. If this conclusion is correct, it appears that the physiological requirements of the two pathogens are different. However, in many parasites, pathogenicity is related to transmission dynamics (May and Anderson 1983, Toft and Karter 1990) and transmission requirements may also be important aspects for pathogenicity of these oyster parasites. Unfortunately, transmission dynamics are poorly understood for both *H. nelsoni* and *P. marinus*.

The absence of *P. marinus* during 1988 in the stocks deployed in 1988 (stocks 1-3) suggests that oyster size may be more important for infectivity of *P. marinus* than of *H. nelsoni*. All three stocks deployed in 1988 acquired *H. nelsoni* during the first summer; oyster sizes ranged from about 12 to 30 mm shell height. None of these stocks acquired *P. marinus* until the second summer when they were approximately 30 to 50 mm in size. The stocks

exposed initially in 1989 (stocks 4-6) all acquired *P. marinus* during the first summer of exposure, but these oysters were all larger than those initially exposed in 1988. On the basis of these results, oysters appear to become infected with *H. nelsoni* at a smaller size than for *P. marinus* infections. However, infectivity of both parasites undoubtedly varies with density of infective stages (dosage), so oysters smaller than 30 mm shell height may become infected with *P. marinus* if a higher density of infective stages are present than occurred during 1988. Crosby and Roberts (1990) found no relationship between *P. marinus* intensity and oyster size, but all oysters examined in their study were greater than 67 mm shell height.

Growth

All stocks in this study became infected with *P. marinus*, so growth between infected and uninfected stocks could not be compared; only relative growth among the various infected stocks was compared. The growth of both MSX-selected stocks was severely affected by *P. marinus* infections. Neither stock reached present

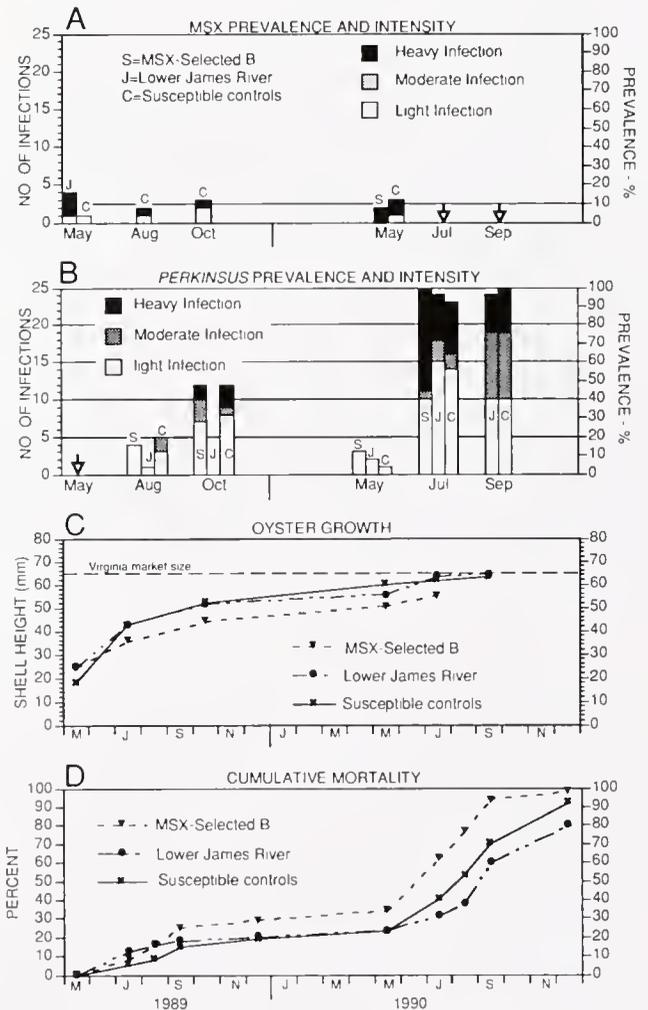


Figure 2. Results from the three oyster stocks deployed in May 1989. In A and B total bar height is the number of infected oysters (left axis) and prevalence (right axis) in a sample of 25 oysters. Arrows indicate samples examined, but no infections found. Oyster stock designations shown in A also apply to B. Time line shown in D applies to all figures.

Virginia market size of 64 mm during the study period and the size of both stocks was significantly lower than any other stock at the end of the study. The growth of the MSX-selected A stock virtually stopped after the first infections of *P. marinus* were observed in August 1989. The average shell height of all other stocks reached Virginia market size. It is interesting that the Delaware Bay native stock grew almost as well as the Mobjack Bay stock since Mobjack Bay oysters have a long history of annual exposure to *P. marinus* and the pathogen has only rarely been present in Delaware Bay.

The retardation of oyster growth by *P. marinus* was first reported by Menzel and Hopkins (1955) and later by Andrews (1961). More recently, Paynter and Burreson (1991) demonstrated rapid reduction in growth in oysters selected over 18 generations for fast growth (Paynter and Dimichele 1990) even when the prevalence and intensity of *P. marinus* were relatively low. The results of the present study and those of Paynter and Burreson (1991) suggest that the growth of highly inbred oysters is more affected by *P. marinus* than is the growth of native stocks, whether or not they have a history of exposure to *P. marinus*.

#### Mortality

Mortality was high in all groups, but mortality in both MSX-selected stocks was significantly greater than in the other four stocks. Based on the relative prevalence and intensity of the two pathogens, these MSX-selected stocks experienced low mortality from *H. nelsoni*, but very high mortality from *P. marinus*, especially during the second year of infection. The Mobjack Bay stock appeared to have the lowest mortality from *P. marinus*, but the moderate mortality in this stock from *H. nelsoni* resulted in a total mortality similar to the Delaware Bay native stock. The lower James River stock had lower mortality from *P. marinus* than the susceptible control stock, but there was no significant challenge from *H. nelsoni* during 1990, so the susceptibility of the lower James River stock to that pathogen is unclear.

On the basis of this study, under conditions of relatively low *H. nelsoni* abundance and relatively high *P. marinus* abundance, it does not appear that the MSX-resistant oyster stocks developed by Rutgers University offer any hope for immediate rejuvenation of the Chesapeake Bay oyster industry. Only two strains were tested, but these resistant oysters seem to be extremely susceptible to *P.*

*marinus*; mortality is very high and growth, after infection, is very low. Growth is important because this study again demonstrates that high mortality from *P. marinus* does not occur until the second summer of infection and, thus, oysters that reach market size in less than two years may avoid significant disease-induced mortality. For example, if hatchery-reared spat were deployed to grow-out areas in the fall and reached market size in 18 months they would experience only one summer of disease exposure. If these oysters had also been selected for decreased susceptibility to both *H. nelsoni* and *P. marinus*, mortality might be relatively low prior to harvest. Acceptable growth and the lowest mortality occurred in the Delaware Bay native stock, the Mobjack Bay stock and the lower James River stock. These stocks, including both direct lines and intraspecific hybridization, will be used in a continuing selective breeding program.

Although the MSX-resistant oyster stocks appear to offer no immediate benefit to the Chesapeake Bay oyster industry, their value could be substantial in the future if *H. nelsoni* returns to its previously dominant abundance and *P. marinus* abates. Intraspecific hybridization between an MSX-resistant stock and a native stock selected for rapid growth and *P. marinus* resistance could yield a strain with decreased susceptibility to both diseases.

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## EFFECTS OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*: II. DISEASE DEVELOPMENT AND IMPACT ON GROWTH RATE AT DIFFERENT SALINITIES

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**ABSTRACT** In order to assess the impact of *Perkinsus marinus* infection on oyster growth and mortality, oysters were raised in floating rafts at six sites around Chesapeake Bay. The sites were comprised of two low salinity sites (8–10‰), two moderate salinity (12–15‰) sites and two high salinity sites (16–20‰). Oyster growth was monitored biweekly along with various water qualities including temperature and salinity. Condition index was measured monthly and disease diagnosis was performed bimonthly. Oyster growth was initially greatest at the high salinity sites but was subsequently retarded by *Perkinsus* infection at both the moderate and high salinity sites (where the parasite was more prevalent). Comparison of pre-infection and post-infection growth rates between sites showed that the reduction in growth rate was mitigated by lower salinity. Condition index was not related to salinity or site but was significantly reduced by *P. marinus* infection. Reduction in condition, however, was not associated with increased mortality. Mortality was also less related to salinity or temperature than it was to infection history (previous infection). Groups which incurred high infection prevalences and intensities exhibited low mortality during their first year, but suffered high mortality during the following year. The results are discussed in relation to management and aquacultural practices and their relation to genetics and selective breeding of disease resistant oysters.

**KEY WORDS:** oyster, growth, *Perkinsus*, disease, mortality, Chesapeake Bay

### INTRODUCTION

Once considered the most abundant source of oysters in the world, the Chesapeake Bay has lost most of its oyster population to the combined effects of disease and overharvesting. In the last 100 years, the existing population has been reduced by an estimated 99%. The subsequent loss of biofiltration normally provided by the oysters has been cited as the principal cause of the historical increase in phytoplankton biomass in Chesapeake Bay which has caused widespread eutrophication problems including hypoxia and anoxia (Newell 1988). The effects of two diseases (MSX and Dermo) caused by two parasitic protozoans (*Haplosporidium nelsoni* and *Perkinsus marinus*, respectively) combined with continued intense harvesting pressure over the last three decades have depleted the natural population to this critically low level (Hargis and Haven 1988).

Infections of *P. marinus* have been documented in Chesapeake Bay oyster populations since the early 1950s (Andrews 1988), and the continued susceptibility of the population to the disease has puzzled scientists over the last two decades. Several oyster strains tolerant of other protozoan infections have naturally evolved (Sindermann 1977) or been developed by breeding programs (Ford and Haskin 1987), so the potential for the development of disease resistance is present in the species. The lack of development of resistance or tolerance to *H. nelsoni* or *P. marinus* by the oyster population in the Bay suggests that unselected susceptible oysters

living in low salinity areas are the major contributors to annual spawn and recruitment and/or that additional pressures may have compromised the species ability to adapt to disease pressure. Pollution has been shown to affect oyster immune systems (Anderson 1988) and may have insulted the oyster population further. However, continued intense harvest pressure on the natural stocks has probably limited the oyster population's ability to develop resistance to the diseases, especially if sublethal effects of infection have caused resistant animals to be harvested before susceptible animals.

Significant sublethal effects of *H. nelsoni* have been demonstrated by a number of studies. These effects include reduction in clearance rates and condition index (Newell 1985) and reduction in other physiological parameters (Barber et al. 1988a, 1988b, Ford and Figueras 1988) including fecundity. However, few studies have examined the sublethal effects of *P. marinus* infection. If significant sublethal effects occurred which resulted in resistant individuals being harvested in disproportionate numbers compared to susceptible animals, then resistance would most likely not evolve or would be slow to evolve. The inhibition of growth in susceptible oysters might cause such a disproportionate harvest and, since the effect is sublethal, would lead to higher contributions by susceptible animals in the next years spawn and recruitment.

The decline of the natural population of oysters in Chesapeake Bay has caused an increased interest in oyster aquaculture in the region. Oyster culture techniques other than traditional remote setting methods have begun to receive more attention. Intensive oyster cultivation in suspended culture has been shown to promote rapid oyster growth (Paynter and DiMichele 1990); however,

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Menzel and Hopkins (1955) and Andrews (1961) have shown that *P. marinus* infection greatly reduces oyster growth and may compromise the advantages of suspended culture in Chesapeake Bay. While the previous studies (Menzel and Hopkins 1955, Andrews 1961) showed clearly that growth reduction was associated with *P. marinus* infection, genetic differences among individuals was not assessed and parasite diagnoses were performed only at the end of the experiment so time of parasite acquisition was unknown. In order to make a more complete assessment of the effects of *P. marinus* on the growth and mortality of whole oyster populations, we initiated a study utilizing large numbers of genetically equivalent oysters raised at different salinities in Chesapeake Bay. Test growouts at several sites that represented different salinity regimes and parasite abundance were established to assess the possible sublethal effects of infection on growth and condition of oysters since infection intensities and disease related mortalities caused by *H. nelsoni* and *P. marinus* have been related to salinity (Ford 1985, Soniat 1985, Burreson and Andrews 1988, Gauthier et al. 1990). Oyster growth, condition and infection status were monitored regularly at all sites. *H. nelsoni* was not detected in any oysters during the two year study; *P. marinus* prevalence, however, was high at several sites. Infection severely inhibited growth in oysters at high and moderate salinities. Mortality during the second year of infection was determined more by history of infection in the first year than by immediate infection intensity.

#### MATERIALS AND METHODS

Oyster spat were introduced to six sites representing different salinities typical of Chesapeake Bay in order to assess the relative impact of *P. marinus* infection on growth and condition in the oysters. Cultchless oysters were produced from selectively inbred broodstock (see Paynter and DiMichele 1990, Brown and Paynter 1991) using traditional hatchery methods. Depending on the experiment, oysters from 10 to 25 mm in shell height were introduced in floating rafts within 2 days at all sites. Rafts were constructed of wooden frames with polyethylene mesh (1.9 cm) folded into a rectangular box which hung below the wooden frame and was stapled to the wooden frame along the edges. The resulting mesh box was 91 cm long  $\times$  61 cm wide  $\times$  20 cm deep. A 90 cm  $\times$  60 cm panel of extruded styrofoam was placed underneath the wooden frame to keep the tray afloat. A polyethylene mesh cage inserted into the tray was used to hold small animals until they were large enough to be held on the 1.9 cm mesh. Approximately 1000 oysters were initially placed in a single tray. If a tray became crowded the group was split into another tray.

The Maryland (MD) sites were comprised of two low salinity sites (8–12‰; Wye River and Deep Cove Creek) and two moderate salinity sites (12–15‰; Worlds End Creek and Slaughter Creek). The Virginia (VA) sites (VIMS and Mobjack Bay) had salinities of 16–20‰ and were denoted as high salinity sites in this study. Typically a site was a shallow tidal creek, well protected from weather and boat traffic. The sites were removed from any point source pollution such as marinas and sewage outlets. Horizontal water flow as judged by casual observation was low at all sites except VIMS. Many of these factors are known to affect bivalve growth, especially water flow, and their effects have been neutralized by the selection of sites with similar characteristics. The VIMS site, which had greater horizontal water flow and was exposed to more open-water conditions (greater wave action), however, showed no differences in growth rate compared to the site of similar salinity in Mobjack Bay.

Growth as shell height was measured every two weeks. Twenty five to fifty oysters were removed from a tray *en masse*, measured to the nearest mm with a ruler and returned to the tray. Length, total weight, shell weight, wet tissue weight, and dry tissue weight were measured monthly throughout the study period in five animals from each tray at each site. Condition indices were calculated from that data as:

$$CI = \frac{\text{dry wt (g)}}{(\text{total wt(g)} - \text{shell wt(g)})}$$

Bimonthly, 25 animals from each tray at each site were removed for parasite diagnosis. Diagnosis of *P. marinus* was by thioglycollate culture of rectal, gill and mantle tissue samples (Ray 1952). Based on Ray (1954) and Mackin (1962), infection intensities were rated and, for calculation of weighted incidence (WI), assigned numerical values as follows: negative = 0, light = 1, moderate = 3, and heavy = 5. WI was calculated as the average value of infection intensity for a sample of twenty five oysters. Diagnosis of *H. nelsoni* was by routine paraffin histology of tissue fixed in Davidson's AFA.

Animals were first introduced to all sites in late July 1989. Additional animals from the same spawn were maintained in floating rafts at the Wye River site where parasite prevalence was zero. Growth, infection status, and condition index were measured through November 1989. Because the initiation of the experiment was in late summer of 1989 and because the experimental trays were lost at the moderate salinity sites over the winter, a second set of oysters was introduced from the Wye River stock to all sites in May 1990 and were monitored until November 1990. This effort provided a more complete assessment of disease onset and its effects.

In an extension of the original growout experiments, additional introductions were made from the Wye River stock to the Mobjack Bay site which exhibited high *P. marinus* prevalences. At that site, where the initial introduction (Group A) was in late July 1989, a second introduction of *P. marinus* free animals (Group B) from the same spawn was made in early September 1989. Additionally, a third group of oysters (Group C) from that spawn was introduced in early May 1990. The subsequent introductions were made to compare the relative infection rates and disease impacts for introductions at different times of the year.

Earlier studies (Paynter and DiMichele 1990, Paynter and Maloney 1991, Paynter, unpublished observations) have shown that oyster shell height increases at a constant rate under the culture conditions described above throughout the growing season. Growth rate, measured as increase in shell height, does not decrease as the animals grow, even when the group triples its original size during a single growing season. Linear regression offers a highly precise estimate of growth rate in a given oyster group at a given site. Therefore, data were treated using linear and polynomial regression to estimate pre- and post-infection growth rates. Analysis of variance (ANOVA) was used to distinguish among salinity, seasonal (monthly) and parasite contributions to the variance of condition indices. ANOVA, regressions and comparisons of  $\beta$  coefficients generated by regression were conducted according to Sokal and Rohlf (1981).

#### RESULTS

*Haplosporidium nelsoni* (MSX) was not detected in any oysters during the two year study described here. *Perkinsus marinus* was the only pathogen detected during the entire study. For clarity of

presentation, data from three (Mobjack Bay, Worlds End Creek, and Wye River) of the six sites will be presented. Trays were lost at Slaughter Creek during the winter months, and data from the VIMS and Deep Cove sites were essentially identical to the Mobjack and Wye River sites respectively.

Infection by *P. marinus* occurred in experimental groups raised at moderate and high salinities, but not at low salinity sites. In August 1990 at both moderate and high salinities, infection prevalences and intensities were equal and relatively low (Fig. 1). However, during the following months both prevalence and intensity of infection increased at high salinities while infection at moderate salinities remained unchanged (Fig. 1).

Growth as shell height was constant with respect to time at all sites in uninfected oyster populations (Fig. 1). Linear regression offered a highly precise estimate of growth rate. Oyster growth rates were higher at high salinity sites compared to growth at low salinity sites ( $P < 0.05$ ; Table 1). The regression of pre-infection growth at moderate salinities was less precise due to the paucity of data collected before infection at both sites. Average oyster growth was severely inhibited as soon as a population became infected, even when the intensity of infection was very low (Fig. 1, Fig. 2, Fig. 3). This effect was quantified by linear regression of pre- and post-infection growth curves (Table 1). This analysis showed that growth rates at high and moderate salinities were significantly reduced after *P. marinus* infection ( $P < 0.001$ ; Fig. 1C; Table 1). Oysters grown at low salinity did not become infected and continued to grow at a constant rate throughout the growing season (Fig. 1A).

In the oyster groups sequentially introduced at Mobjack Bay during 1989, Group A was infected within 45 days of introduction, and growth in that group appeared to be reduced (Fig. 2) as a result. While linear regression over the entire time period yielded a highly significant relationship ( $r^2 = .94$ ), treating the data as having a distinct break in the growth curve associated with the detection of disease yielded even greater significance ( $r^2 > .98$  for both lines). Group B was infected in October 1989 but infection was not detected in November 1989. Growth in Group B did not appear to be affected by infection in October, however winter temperatures stopped growth before enough data could be collected to make a comparison of pre- and post-infection growth. No unusual mortality occurred in 1989 in any of the groups at any sites.

No infections were detected in May 1990 in groups that had become infected during 1989. Latent infection or misdiagnosis seems unlikely because the oysters grew well until infection was detected, and only then did the oysters exhibit a marked reduction in growth rate (Fig. 3, Table 2). In the two groups held over from 1989 at Mobjack Bay (Groups A & B), significant mortalities occurred only in the group that had been infected and exhibited reduced growth in 1989 (Group A: Figs. 2 & 3). When Group B became infected and had equivalent intensity of infection as Group A, mortality was comparatively low (Fig. 3). Group A was diagnosed with 20% heavy infections in November 1990 while Group B had only 8% heavy infections.

Three factor ANOVA was used to differentiate among the effects of site, month, and infection status on condition index (CI). Oyster groups were categorized as infected or uninfected for the analysis. CI was not different among sites in uninfected or infected oyster groups. However, CI was closely related to month and was significantly lower in infected groups compared to uninfected groups (Fig. 4) regardless of site. Condition was not significantly

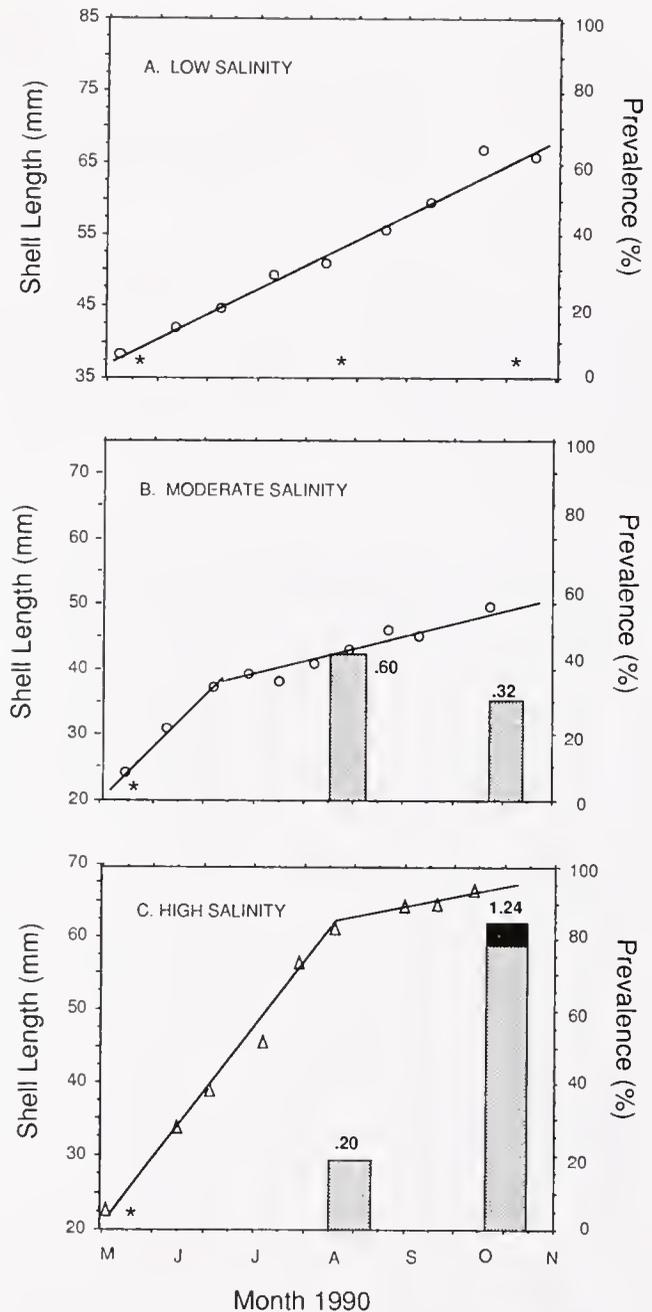


Figure 1. Increase in shell height and disease prevalences over time at three different salinities during 1990. A. low salinity (8–12‰), B. moderate salinity (12–15‰), and C. high salinity (16–20‰). Disease prevalence as percent of the animals sampled is shown by the columns (axis on right side). Numbers above bars in the prevalence graph are weighted incidences of infection (see text) and shaded portions of the bars represent percent of animals with heavy infections. Asterisks (\*) indicate oysters were examined with no parasites found.

different between Mobjack groups A & B during any months even though A had significantly greater mortality than B.

The effects of infection were related to salinity in two ways: 1) *P. marinus* was not detected at low salinities (Fig. 1A), and 2) the intensity of infection and subsequent reduction in growth rate was not as great at moderate salinities (Fig. 1B) as at high salinities (Fig. 1C). Growth rates decreased from approximately 14 mm/

TABLE 1.

Effect of *P. marinus* infection on oyster growth rates at different salinities.

Salinity	Pre-infection	Post-infection	% Reduction
High (16–20‰)	14.34 (1.2)	2.85 (.39)	80
Moderate (12–15‰)	9.45 (1.7)	3.84 (.45)	60
Low (8–10‰)	7.86 (.33)	N.I.	N.I.

Growth rates were calculated by performing linear regression on shell height over time and are presented in mm/month. All regressions were highly significant ( $P < .005$ ) ( $r^2 > 0.95$  pre-infection). Numbers in parentheses are standard errors of the estimate (S.E.E.). Infections at high and moderate salinities were associated with significantly reduced growth rates ( $P < 0.05$ ). Pre-infection growth rates were significantly higher at the high salinity sites compared to the low salinity sites ( $P < 0.05$ ). N.I. = not infected.

month to nearly 2.85 mm/month at the high salinity site after infection occurred. At the moderate salinity site the pre-infection growth rate was 9 mm/month and declined to approximately 3 mm/month (Table 1). Time to infection after introduction of the 1990 groups ranged from as early as 45 days to as long as 120 days and was quite variable between sites and salinities. For instance, infection was first detected at the Slaughter Creek site (a moderate salinity site) within 45 days of introduction while infection at the VIMS site occurred within 75 days and at Mobjack Bay within 120 days. Time to infection of the larger animals (Groups A & B) at

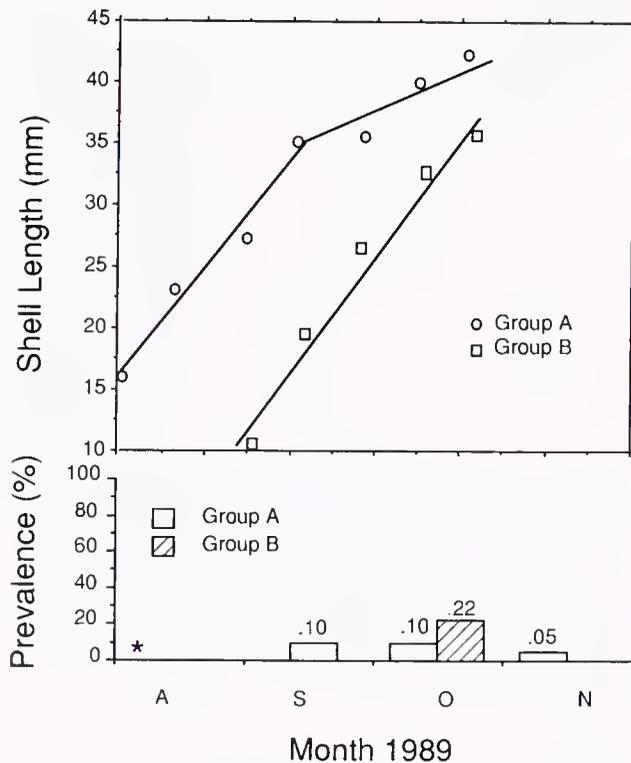


Figure 2. Growth and infection profile of two groups of oysters from the same population introduced at the Mobjack Bay site in 1989. Animals were monitored from August (A) through November (N). Group A was introduced in July 1989 and Group B in September 1989. Numbers above bars in the prevalence graph are weighted incidences of infection (see text). Asterisks (\*) indicate oysters were examined with no parasites found.

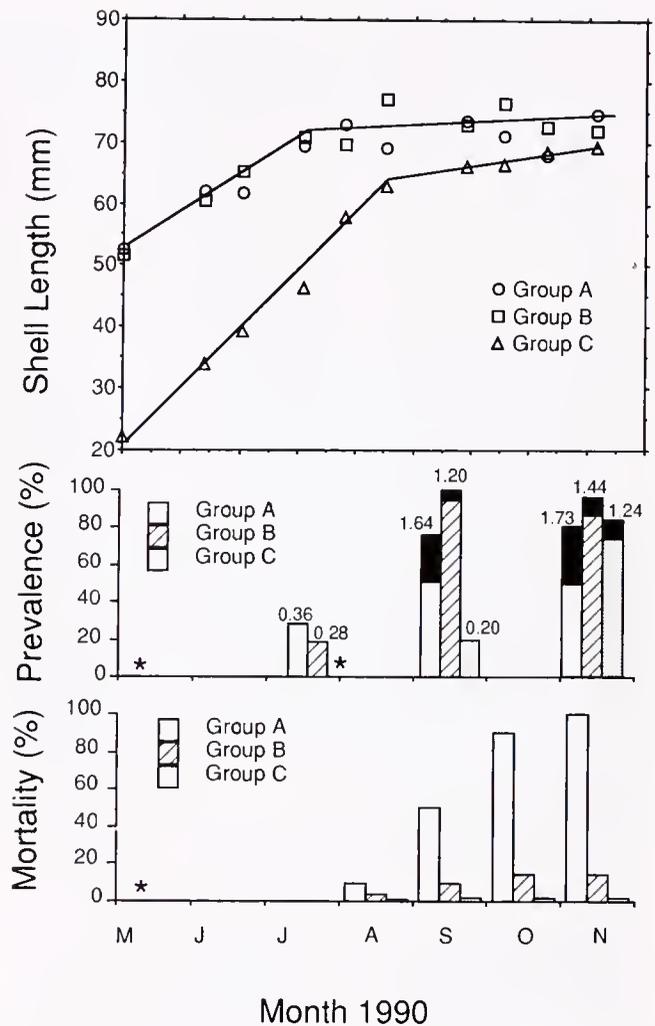


Figure 3. Growth, infection profile, and cumulative mortality during 1990 of three groups of oysters from the same population introduced at the Mobjack Bay site at different times. Animals were monitored from May (M) through November (N). Group A was introduced in July 1989, group B in September 1989, and group C in May 1990. Numbers above bars in the prevalence graph are weighted incidences of infection (see text) and shaded portions of the bars represent percent of animals with heavy infections. Mortality expressed in cumulative percent. Asterisks (\*) indicate oysters were examined with no parasites found.

Mobjack Bay was shorter than in the smaller animals (Group C; Fig. 3).

## DISCUSSION

This study confirms the observations made by Menzel and Hopkins (1955), Ray et al. (1953), and J. D. Andrews (1961) over three decades ago: infection by *P. marinus* results in the severe retardation of growth in oysters. However, those studies were conducted on individual oysters which prevented the assessment of infection status in the animals except at the end of the experiment or when an oyster died. Therefore, the time of initial infection, the relationship of infection intensity to growth reduction, or the effect of infection on large populations of oysters could not be addressed. Interestingly, however, the measurement of growth in the present and related studies (Paynter and DiMichele 1990, Paynter and Mallouze 1991), which used shell height as a measure of growth

TABLE 2.  
Size distribution of oysters raised at the Mobjack Bay site.

Date	Group	Mean Ht	Variance	S.E.M.	Min	Max
5/31/90	A	61.97	79.76	0.30	45	87
	B	60.32	128.04	0.26	40	90
	C	34.00	19.60	0.12	25	45
7/4/90	A	69.43	56.17	0.33	55	85
	B	70.83	148.06	0.53	54	92
	C	46.29	40.61	0.30	38	57
8/29/90	A	73.55	46.07	0.31	62	89
	B	72.95	78.05	0.42	54	88
	C	66.04	36.22	0.25	56	78
9/26/90	A	67.94	45.40	0.22	58	82
	B	72.48	84.18	0.34	59	92
	C	68.47	98.00	0.31	45	85

Oysters were sampled as described in text.

and subsampled large groups of genetically equivalent oysters, has yielded results remarkably similar to Andrews underwater weighing technique, and has shown that oysters grow at a relatively constant rate throughout the warm season (Menzel and Hopkins 1955, Andrews 1961).

The characterization of the effects of *P. marinus* on large numbers of animals and the influence of salinity on those effects is important to the developing aquaculture industry. However, few studies have focused on sublethal or physiological effects of protozoan parasitism in bivalves (see Newell and Barber 1988). Barber et al. (1988a, 1988b) showed that condition and fecundity were reduced by infection of *Haplosporidium nelsoni* (MSX). Newell (1985) showed that *H. nelsoni* infection caused significant reduction in clearance rate and condition index but was not related to oxygen consumption or growth cessation. Unfortunately, *P. marinus* infection was not tested in that study, and the physiological effects of *P. marinus* infection remain, for the most part, uninvestigated. This study, however, provides insight into several aspects of the physiological impact of *P. marinus* infection on *C. virginica*. Briefly, we have quantified the retardation of growth induced by infection in whole populations of oysters, shown that disease-related mortalities were low until the second year of infection, and that infection was correlated with reduced condition index. Finally, salinity was inversely correlated with the intensity of disease development and subsequent reduction of growth rate, but not the time to infection or the effect of infection on condition

index. These results suggest that the concentration and/or virulence of infective *P. marinus* life stages is higher at higher salinities (Chu and Greene 1989, Chu and La Peyre 1991), and/or that the immunological and physiological characteristics of oysters with respect to disease tolerance are more robust at lower salinities (Fisher and Newell 1986).

The reduction in growth rate after *P. marinus* infection was essentially immediate within a tray at a given site (Figs. 2 & 3). Once infection occurred, the entire population within the tray appeared to be affected. For instance, growth in group A (Fig. 2) during 1989 was reduced when the detected prevalence was low ( $\approx 12\%$ ) with low intensity through November. Oysters grown at moderate salinity showed over a 60% reduction in growth rate (Table 1) but incurred only a 30 to 40% infection prevalence throughout the season (Fig. 1). The lack of an increase in size variation or range within infected trays (Table 2), which would be expected if only a small percentage of the animals stopped growing, also suggested that most of the animals within the tray had stopped growing. The data in Table 2 show that the mean size of oysters did not significantly increase after infection (detected on 7/4/90 for Groups A & B) and that the variance of the samples decreased greatly (from 148 to 84 for Group B). In addition, the range of sizes did not change indicating that very few, if any, oysters continued to grow.

During part of the study, disease diagnosis was conducted on oysters whose size and wet tissue weight were also measured. Within a sample for a given tray, no relationship between size and infection was found. When data were pooled from all trays exposed for the same period at a given site, a weak but significant positive correlation was discovered ( $P < 0.05$ ; data not shown). The larger animals tended to acquire the disease sooner and more intensely. This relationship was also evident in Fig. 3 which shows that the smaller group (C) did not become infected until nearly 40 days after groups A or B. Although disease was not detected in any groups in May 1990, latent infections which did not affect growth could have been present in Groups A & B and led to earlier infections in those groups. Crosby and Roberts (1990) found no relationship between size and infection, however, our findings support the generally accepted theory that small oysters or spat are less likely to become infected due to the limited volume of water they filter compared to large animals. However, in this study all oysters within a tray appeared to be affected even before 100% prevalence could be detected. This observation could be the result

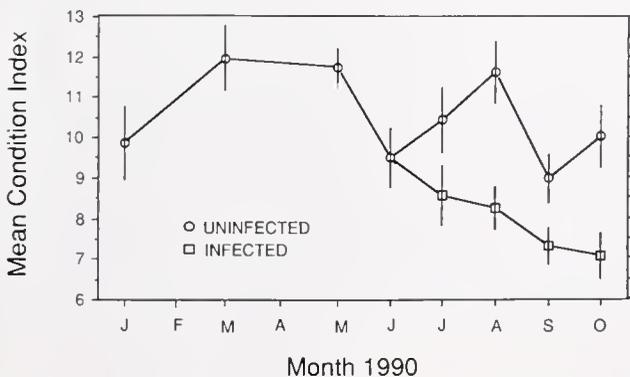


Figure 4. Mean condition indices (CI) of infected and uninfected oysters during 1990 in all groups at all sites by month. Bars represent  $\pm$  S.E.M.

of problems in the diagnostic technique which resulted in misdiagnosis of very light infections (see Gauthier and Fisher 1991); it could suggest that the reduction in growth rate is part of the oyster's response to the detection of an initial superficial infection or irritation; or it could be the result of the mortality of larger oysters only. This final hypothesis is supported by the data in Table 2 which shows that the maximum size stops increasing with infection but the minimum continues to increase albeit at a lower rate. The size of dead oysters was unfortunately not recorded during this experiment.

Mortality was related less to infection intensity, temperature or salinity than it was to previous infection. Group A oysters grown at the Mobjack Bay site (Figs. 2 & 3) acquired infections during 1989, yet no increased mortality was observed. Infections in Group A were lost or reduced over the winter and the oysters were equivalent to Group B in size and condition in May 1990 (Fig. 3). During 1990 the animals in Group A grew at the same rate as the animals in Group B, were infected at the same time, acquired equivalent infection intensities, and showed the same reduction in growth rate and condition index. However, significant mortality (100%) occurred in Group A but not in Group B (Fig. 3). Group C was infected later, as mentioned above, theoretically due to its smaller size, and did not suffer increased mortality. It is important to remember that all three groups were from the same spawn and therefore were genetically equivalent. These results are similar to those found by Burreson (1991) in other oyster strains, and they suggest that as yet undetectable injury was caused by *P. marinus* infection which made oysters which had been previously infected more vulnerable to developing terminal infections. Interestingly, the weighted incidences of the oyster groups which had large mortalities were lower than previous experience would have predicted. This may indicate that the selectively inbred oyster population is more susceptible to *P. marinus*-induced mortality than more resistant native oyster populations (Burreson 1991).

Another important physiological impact on oysters infected with *P. marinus* was on condition index (CI). Interestingly, condition index was more a function of month or season than site. This suggests that CI is regulated by temperature and its effects on seasonal biological cycles (i.e. spawning) and not by salinity. Three-factor ANOVA showed that CI was significantly reduced by infection (Fig. 4). These findings are in general agreement with those of other studies; however, Craig et al. (1989) found that CI varied greatly between sites along the Gulf coast. Furthermore, that study found a negative correlation between salinity and CI, but was unable to distinguish between the effects of salinity vs. *P. marinus* infection. Gauthier et al. (1990) showed that *P. marinus* infection was closely associated with reduced condition in oysters along the Louisiana coast. The results for Chesapeake Bay presented in this report clearly show that CI is not affected by salinity or site and that infection has a substantial negative impact on CI. Crosby and Roberts (1990) found similar relationships in oyster populations in South Carolina. Mortality was not associated with a further reduction in CI (i.e. the mean CI of group A, Fig. 2, was not significantly lower than the CI of the animals of group B during infected periods). It appears from this data that while CI is reduced by infection, susceptibility to infection or mortality caused by infection are not related to CI.

Salinity was positively correlated with growth rate of oysters (Fig. 1). Unfortunately, the increase in growth rate was compromised by the increased prevalence and effects of *P. marinus* at higher salinities. It is therefore important to understand the relationship between infection, its effects, and salinity. This study has shown that infection rate, measured as time to infection, was not related to salinity but probably more to the number of infective stages present in the water. Quick and Mackin (1971) found little relationship between salinity and infection. Soniat (1985) and Ragone and Burreson (1990), on the other hand, found a significant correlation between salinity and infection. The findings of the present study are compatible with both sets of studies. While little difference in initial infections was found between high and moderate salinities, no infections occurred at low salinities. In relative terms, the impact of infection appeared positively correlated with salinity. Growth in oysters infected at moderate salinities was reduced by an average of 60% compared to a reduction of 80% at higher salinities. Unfortunately, mortality at moderate salinities could not be quantitated due to the loss of experimental trays at those sites during the second year of study. However, the absolute values of the growth rates of infected oysters were not different between salinities indicating that the relative differences were a function of pre-infection growth rate and that the growth rates of all infected populations, regardless to salinity, declined to the same low (approximately 3 mm/month) level.

Finally, the selectively inbred population employed in this study and the MSX-resistant strains employed in a closely related study (Burreson et al. 1990, Burreson 1991) were both more affected by *P. marinus* infection than either Mobjack Bay native or Delaware Bay native animals. This suggests that the results of the present study may not be representative of other oyster populations, but also indicates that a genetic component of disease resistance exists in the species which might be enhanced by selective breeding. Significant evidence supports the opinion that genetically distinct populations of *C. virginica* exist along the Atlantic and Gulf coasts (King and Gray 1990, Reeb and Avise 1990, Brown and Paynter 1991). It is possible that the genetic differences defined by these studies are reflective of physiological differences which may be important to identify. For instance, the southern "race" of oysters as identified by Reeb and Avise (1990) has probably been exposed to *P. marinus* for many more generations than the northern race. It is entirely possible that the southern oyster population would exhibit more tolerance to infection than the Chesapeake Bay native populations. Transplantation experiments to test this and related hypotheses are currently underway.

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## SUSCEPTIBILITY OF DIPLOID AND TRIPLOID PACIFIC OYSTERS, *CRASSOSTREA GIGAS* (THUNBERG, 1793) AND EASTERN OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791), TO *PERKINSUS MARINUS*

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**ABSTRACT** The susceptibility of Pacific oysters, *Crassostrea gigas*, to the oyster parasite *Perkinsus marinus* was compared with that of eastern oysters, *Crassostrea virginica*, in two separate experiments. Experiments were conducted in flow-through seawater systems with quarantined effluent. Oysters were challenged by addition of infective *P. marinus*. In the first experiment, which used only diploid oysters, 40% of *C. gigas* became infected with *P. marinus* after 83 days compared to 100% of *C. virginica*. In the second experiment, which examined susceptibility of diploid and triploid individuals of both species, prevalence was high in all groups after 60 days. In *C. virginica*, heavy and moderate infection intensities prevailed while *C. gigas* exhibited only light infections. Cumulative mortality of *C. virginica* after 150 days was 100% for the diploid group and 97.7% for the triploid group. Cumulative mortality of *C. gigas* after 150 days was 25.1% for the diploid group and 34.3% for the triploid group, but this mortality did not appear to be disease related. Thus, *C. gigas* was consistently more tolerant of *P. marinus* than was *C. virginica*, and triploidy provided no increased disease tolerance for either species.

**KEY WORDS:** disease, ploidy, Chesapeake Bay, oyster industry

### INTRODUCTION

*Perkinsus marinus* (Mackin, Owen and Collier) is a serious pathogen in the eastern oyster, *Crassostrea virginica* (Gmelin, 1791). The parasite is widely distributed in oyster populations from the Gulf of Mexico north into Delaware Bay. In Chesapeake Bay, *P. marinus* was originally confined to the lower portions of the Bay, but in recent years has spread into all of Virginia's oyster beds (Andrews 1988) where it has replaced *Haplosporidium nelsoni* (Haskin, Stauber and Mackin), popularly known as MSX, as the most important oyster pathogen (Andrews 1988, Burreson and Andrews 1988).

Since reaching its peak of 7.6 million bushels in 1904, production of market oysters in Virginia has steadily declined. During the past 30 years mortality resulting from the combined effects of *P. marinus* and *H. nelsoni* have played a substantial role in this decline. Recent harvest levels have been well below 500,000 bushels annually (Hargis and Haven 1988) with record low landings each of the last three years. The 1990 oyster harvest in Virginia yielded only 135,704 bushels of market oysters.

Among the options being considered to revitalize Virginia's oyster industry is the use of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg, 1793), in conjunction with *C. virginica* as the basis for oyster production. One method of reducing losses of oyster stocks to disease is to utilize oyster species that are not susceptible to local diseases. In some areas of Europe, notably France, the loss of endemic oyster species to disease has led to their supplementation or replacement with *C. gigas*. For example, in the 1970s, France's native populations of the Portuguese oyster, *Crassostrea angulata*, were decimated by two viral diseases, gill necrosis virus (GNV) and hemocytic infection virus (HIV). By 1983 *C. angulata* had disappeared from French oyster beds. *Crassostrea gigas* appears to be highly resistant to both GNV and HIV (Comps 1988). Major imports of *C. gigas* to France in the 1970s

prevented the collapse of the oyster industry (Comps 1988, Chew 1990). *Crassostrea gigas* has also demonstrated resistance to the protozoan parasites *Bonamia ostreae* and *Marteilia refringens* (phylum Ascomycota). Both these parasites cause severe pathology and serious recurrent mortality in the edible oyster, *Ostrea edulis* (L.) in France. Cells resembling early stages of *M. refringens* can be found in *C. gigas* grown in areas where this parasite is endemic, but there is no associated pathology or mortality in this oyster species (Figuera and Montes 1988). In France, significant mortality in *O. edulis* resulting from *M. refringens* prompted supplemental plantings of *C. gigas* to help offset losses in production (Cahour 1979). *Bonamia ostreae* is found worldwide in *O. edulis*, but infections in *C. gigas* are unknown (Chagot et al. 1989). Thus far, *C. gigas* has demonstrated low susceptibility to viral diseases and to ascetosporan parasites; however, its resistance to *Perkinsus*-like parasites (phylum Apicomplexa) has yet to be demonstrated.

The use of triploid oysters is another option to revitalize Virginia's oyster industry. Triploid oysters, having an extra set of chromosomes, exhibit reduced gametogenesis, greater glycogen content and faster growth than diploid oysters (Stanley et al. 1984, Allen and Downing 1986, 1990). Thus, triploid *C. gigas*, by virtue of reduced gametogenesis, may be advantageous because they offer the potential for utilizing a non-native species in Chesapeake Bay while reducing the risks of unwanted introductions of reproductively competent individuals. Triploid *C. virginica* may also be advantageous, but for different reasons. Diversion of energy from gametogenesis to other uses in triploid *C. virginica* may be reflected as increased disease tolerance if more energy is allocated to defense mechanisms. In any case, triploid *C. virginica* represent a potential advantage over diploid individuals because they may grow faster and reach marketable size before incurring large losses to disease (Barber and Mann 1991).

Determining susceptibility to disease is the first step that must be taken in the evaluation of *C. gigas* as a supplemental species in Virginia waters. The experiments described below examine the comparative susceptibility of diploid and triploid *C. gigas* and *C. virginica* to *P. marinus*.

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## MATERIALS AND METHODS

### Experiment 1

This preliminary experiment was designed to determine if adult *C. gigas* were susceptible to *P. marinus*. On 6 June 1989, 200 *C. gigas* measuring 38–51 mm (1.5–2.0 inches) shell height were obtained from Coast Oyster Company, Quilecene, Washington. Because of unusually low salinity (11.0 ppt) at this time at the Virginia Institute of Marine Science (VIMS), Gloucester Point laboratory, the oysters were placed in a flowing sea water flume at the VIMS Eastern Shore laboratory at Wachapreague, Virginia and allowed to acclimate for six days prior to challenge with *P. marinus*. On 12 June, 83 *C. gigas* were placed into each of two flumes and 25 *C. virginica* were interspersed among the *C. gigas* in one of the flumes for *P. marinus* challenge. The *C. virginica* were obtained from Deep Water Shoal in the upper James River, a bed that is monitored monthly for *P. marinus*. Oysters at this site have only rarely been infected with *P. marinus*, always in late fall. A sample of 25 oysters from the June collection was negative for *P. marinus*. Oysters from Deep Water Shoal were acclimated to the higher Wachapreague salinity in two steps over 96 hours. At the start of the experiment water temperature was 23.7°C and salinity was 32 ppt in both flumes.

Oysters from Deep Water Shoal were also used as negative controls. On 12 June, 25 oysters were placed in an aquarium at VIMS, Gloucester Point, VA filled with 1.0 µm-filtered York River water and maintained at 24°C and 20 ppt. Oysters were fed daily and the water was changed every other day.

Influent water to the flumes at Wachapreague was maintained at a low flow rate (about 7 liters per minute) to prevent rapid flushing of the infectious stages of *P. marinus* and ensure a sufficient exposure period. To compensate for the decreased amount of incoming food, one liter of concentrated ( $2.0 \times 10^5$  cells/ml) wild phytoplankton was added to each flume daily. Phytoplankton was concentrated by passing raw seawater through a 10 µm bag filter and dewatering in a clarifier. Flumes were cleaned weekly just prior to addition of *P. marinus*. Effluent from both flumes was quarantined by passage through a sand filter and discharge onto a gravel lot. Because of the low flow rate, effluent was retained on this lot until it was absorbed.

Oysters in the treatment flume were exposed to *P. marinus* weekly by the addition of either fresh, minced, infected *C. virginica* tissue (Ray 1954a), minced tissue cultured in fluid thioglycollate media, or purified prezoosporangia. Prezoosporangia were purified by culturing minced, infected *C. virginica* tissue in fluid thioglycollate media for three days and then centrifuging to form a pellet of zoosporangia. The pellet was resuspended and washed four times in 0.22 µm-filtered seawater and was resuspended in filtered seawater prior to being added to the treatment flume. One liter of concentrated algae was added to the flume at the time of introduction of *P. marinus* to stimulate oyster feeding and ensure clearance of the parasite. At the same time the control flume received algae only. Influent water to both flumes was then left off for two hours. As an additional source of *P. marinus* challenge, 10 live oysters from Wreck Shoal, a *P. marinus* endemic area in the James River, were also placed in the treatment flume on 3 July. These oysters were marked and interspersed among the other oysters and allowed to die and decay.

The flumes were checked daily and gapers (dead oysters containing tissue) were collected and tested for *P. marinus* using the

fluid thioglycollate assay (Ray 1952). On 28 August, after 45 days of exposure to the parasite, all surviving *C. virginica* (23) and 25 live *C. gigas* from each flume were sacrificed and examined for the presence of *P. marinus*. Samples of 25 *C. gigas* from each treatment were tested again on 9 October, 83 days post exposure. The control oysters from the aquarium were also sacrificed on 9 October and examined for *P. marinus* to assure that the *C. virginica* used in the experiment were not infected prior to being placed in the challenge flume at Wachapreague.

### Experiment 2

In this experiment diploid and triploid *C. gigas* and *C. virginica* were tested for susceptibility to *P. marinus*. *Crassostrea virginica* broodstock was obtained from Mobjack Bay, Virginia and *C. gigas* broodstock was obtained from Coast Oyster Company. All broodstocks were conditioned at 22°C in the VIMS oyster hatchery. Species were spawned separately. Spawning was induced by raising the water temperature to 30°C; all spawnings used gametes from at least 10 individuals. If elevated temperature did not induce spawning, a male oyster from the broodstock group was stripped and a sperm suspension added to the spawning tank. When oysters spawned, they were placed in containers so as to collect sperm and eggs separately. Eggs were fertilized by the addition of sperm. Larvae were reared in 400 gallon conical tanks, set on ground oyster shell (minicultch) and hardened in upwellers. International Council for the Exploration of the Seas (ICES) protocol for handling the non-indigenous *C. gigas* was followed throughout the experiments (ICES 1984). In the hatchery, all effluent was chlorinated and dechlorinated prior to discharge. Diploid *C. virginica* were spawned on 5 April, 1989 and triploidy was induced in *C. virginica* spawned on 23 March; *C. gigas* were spawned on 11 April, 1989 and triploidy was induced in a portion of these embryos. Triploidy was induced by exposing embryos to 1.0 mg/l cytochalasin-B in 1.0 ml dimethyl sulfoxide (DMSO) at 25°C for 15 minutes beginning 15 minutes after fertilization (Downing and Allen 1987). Triploidy of spat was assayed in a sample of 25 oysters by Dr. Stan Allen of Rutgers University using flow cytometry.

Spat were grown in flowing seawater flumes for 14 months allowing them to attain a size of about 40 mm at which time they were large enough to be easily counted and handled. On 18 June, 1990, 175 oysters from each of the four groups were placed in separate Nestier trays in a single outdoor flume. Influent flow rate was high, approximately 20 liters per minute, to provide the oysters with sufficient food and oxygen. Effluent was diverted to a sand retention pond. Flumes were cleaned and the position of the Nestier trays within the flumes was rotated bi-weekly.

For *P. marinus* challenge, oysters infected with *P. marinus* were placed in a waring blender with 1.0 µm filtered seawater and minced by blending with 5 second pulses (Ray 1954a). The minced tissue and several liters of cultured algae were added to the flume after the bi-weekly cleaning. The water was left static for 2 hours to allow for uptake of *P. marinus* by the oysters. The addition of *P. marinus* infected tissue to the flume was discontinued after the first sample revealed that all oyster groups had acquired infections.

At the start of the experiment on 18 June an initial sample of 25 oysters from each group was examined for the presence of *P. marinus* and subsequent samples were examined on 28 August, 26 September, 8 November, 1990, and 3 July 1991. Counts of live and dead oysters were made periodically to assess mortality rates.

For both experiments, infection intensity in gapers and live oysters was rated and assigned a numerical value for calculation of weighted incidence (WI) as negative = 0, light = 1, moderate = 3, and heavy = 5 (Ray 1954b, Andrews 1988). These values were added and divided by the number of oysters examined to give an average infection intensity (weighted incidence) (Mackin 1962).

Differences in prevalence and intensity of *P. marinus* infections and cumulative mortality between the various oyster groups in both experiments were tested by chi-square contingency tables with continuity correction. For prevalence comparisons, the two contingency table columns were numbers of infected and uninfected oysters in the sample. For intensity comparisons, one column of the contingency table was the total number of moderate and heavy infections and the other column was the number of light infections. For mortality comparisons, the two columns were numbers of live and dead oysters to that date. In all comparisons, contingency table rows were the various groups of oysters. All statistical analyses were performed on a Macintosh Hci using Statview II.

## RESULTS

### Experiment 1

Infection prevalence and intensity were higher in *C. virginica* than in *C. gigas*. The first sample, taken 45 days after initial exposure to *P. marinus*, revealed that infection prevalence in *C. virginica* had reached 100% while none of the *C. gigas* were infected. Statistical analysis indicated that both prevalence and intensity of infection was significantly greater in *C. virginica* than in *C. gigas* (Table 1). The second sample of *C. gigas* revealed that 40% of the challenged oysters and 8% of the non-challenged oysters had light infections. The infected oysters in the non-challenged flume apparently acquired infections from the incoming water. No *P. marinus* infections were found in the control oysters held in an aquarium at VIMS, Gloucester Point.

Throughout the course of the experiment 11 *C. gigas* individuals died in the challenge flume and they were all collected as gapers. Of these, two were found to contain *P. marinus*; one oyster contained a single cell and the other oyster had a very light infection. Two *C. virginica* gapers were collected from the challenge flume prior to sampling of all the *C. virginica* on 28 August; one gaper had a light infection and the other had a heavy infection.

Three gapers were found in the *C. gigas* control flume; all tested negative for the presence of *P. marinus*.

### Experiment 2

Throughout the course of this experiment infection intensity, but not prevalence, was much higher in *C. virginica* than in *C. gigas*, regardless of ploidy, and significant differences in infection intensity were found between the two species for all samples (Table 2). No *P. marinus* was found in any group of oysters at the start of the experiment. Samples examined on 27 August revealed that prevalence of *P. marinus* had increased in all groups to between 87 and 100%, but there was no significant difference among the groups. However, infection intensity was significantly higher in both *C. virginica* groups than in either *C. gigas* group (Table 2). As of 27 August, mortality was significantly higher in diploid *C. virginica* than in triploid *C. virginica* even though prevalence and intensity of *P. marinus* infections were similar in the two groups. In subsequent samples examined on 26 September and 8 November, 1990 prevalence of *P. marinus* infections declined in *C. gigas*, but not in *C. virginica*, and differences were statistically significant. The prevalence of *P. marinus* in *C. gigas* had increased by July of 1991 when the experiment was terminated, but all infections were light and no additional mortality had occurred (Table 2). Of the 95 *C. virginica* examined during the experiment, 55 had heavy infections and 24 had moderate infections. No heavy or moderate infections were found in any *C. gigas*. Consequently, weighted incidence in *C. gigas* never reached 1.0, while in *C. virginica* it approached 4.0. There was no difference in prevalence or intensity between diploid and triploid oysters of the same species. However, ploidy assays showed that the *C. gigas* triploid group contained only 67% triploid individuals at the start of the experiment and 36% triploid individuals on 3 July 1991. The *C. virginica* triploid group was 96% triploid at the beginning of the experiment, but no final determination could be made because all *C. virginica* had died by late summer 1990.

Mortality was also much higher in *C. virginica* than in *C. gigas* (Table 2). The highest mortality in *C. gigas* occurred from 7 August to 27 August; during this period mortality was 22.3% in the diploid *C. gigas* and 25.1% in the triploid group. Mortality in both groups of *C. gigas* declined after 27 August and no mortality occurred after 21 September. Cumulative mortality in both diploid

TABLE 1.

Prevalence and intensity of infections in diploid, adult *Crassostrea gigas* and *C. virginica* challenged with *Perkinsus marinus*.

Date	Oyster Group	No. Infected/ No. Examined	% Infected	H-M-L <sup>a</sup>	W.I. <sup>b</sup>
12 Jun 89	<i>C. gigas</i>	0/25	0		0.00
	<i>C. virginica</i>	0/25	0		0.00
28 Aug 89	<i>C. gigas</i> challenged	0/25	0		0.00
	<i>C. gigas</i> control	0/25	0		0.00
	<i>C. virginica</i> challenged	23/23**	100	4-4-15**	2.04
9 Oct 89	<i>C. gigas</i> challenged	10/25	40	0-0-10	0.40
	<i>C. gigas</i> control	2/25	8	0-0-2	0.08
	<i>C. virginica</i> control (aquarium)	0/13	0		0.00

<sup>a</sup> H = number of heavy *P. marinus* infections, M = moderate infections, L = light infections.

<sup>b</sup> W.I. = weighted incidence.

\*\* P < 0.01.

TABLE 2.

Prevalence and intensity of infections in diploid and triploid *Crassostrea gigas* and *C. virginica* challenged with *Perkinsus marinus*.

Date	Oyster Group	No. Infected/ No. Examined	% Infected	H-M-L <sup>a</sup>	Weighted Incidence	Cumulative Mortality
18 Jun 90	<i>C. virginica</i> 2n	0/25	0		0.00	0
	<i>C. virginica</i> 3n	0/25	0		0.00	0
	<i>C. gigas</i> 2n	0/25	0		0.00	0
	<i>C. gigas</i> 3n	0/25	0		0.00	0
27 Aug 90	<i>C. virginica</i> 2n	25/25	100	17-6-2	4.20	48.0%**
	<i>C. virginica</i> 3n	25/25	100	15-4-6	3.72	25.7%
	<i>C. gigas</i> 2n	20/23	87	0-0-20**	0.87	22.3% <sup>b</sup>
	<i>C. gigas</i> 3n	22/25**	88	0-0-22**	0.88	25.1% <sup>b</sup>
26 Sep 90	<i>C. virginica</i> 2n	16/16	100	10-3-3	3.88	76.0%
	<i>C. virginica</i> 3n	25/25	100	10-11-4	3.48	61.1%
	<i>C. gigas</i> 2n	16/25**	64	0-0-16**	0.64	25.1%**
	<i>C. gigas</i> 3n	16/25**	64	0-0-16**	0.64	34.3%**
08 Nov 90	<i>C. virginica</i> 2n	All dead				100.0%
	<i>C. virginica</i> 3n	4/4	100	3-1-0	4.50	97.7%
	<i>C. gigas</i> 2n	2/25**	8	0-0-2*	0.08	25.1%**
	<i>C. gigas</i> 3n	1/25**	4	0-0-1*	0.04	34.3%**
03 July 91	<i>C. virginica</i> 2n	All dead				100.0%
	<i>C. virginica</i> 3n	All dead				100.0%
	<i>C. gigas</i> 2n	5/25	20	0-0-5	0.20	25.1%**
	<i>C. gigas</i> 3n	3/25	12	0-0-3	0.12	34.3%**

<sup>a</sup> H = number of heavy *P. marinus* infections, M = moderate infections, L = light infections.<sup>b</sup> Mortality in *C. gigas* not disease related, see Discussion.

\* P &lt; 0.05.

\*\* P &lt; 0.01.

and triploid *C. virginica* increased steadily during the experiment. At the time the last count was taken, 150 days post exposure, cumulative mortality had reached 100% in diploid *C. virginica* and 97.7% in the triploid group. Cumulative mortality was significantly higher in both *C. virginica* groups than in either *C. gigas* group after 26 September (Table 2).

#### DISCUSSION

These results suggest that *C. gigas* is much more tolerant of *P. marinus* than *C. virginica* and also that triploidy provides no increased tolerance for either species. In the first experiment, which examined only diploid oysters, *P. marinus* infections appeared in *C. virginica* sooner than in *C. gigas* suggesting that *C. gigas* may be less susceptible to infection. Since all of the oysters used in this experiment were similar in size and were interspersed in the same tank, they would be expected to acquire infections at the same rate if they were equally susceptible.

Prevalence and intensity of *P. marinus* infections were lower in *C. gigas* than in *C. virginica* in both experiments and in the second experiment prevalence of *P. marinus* actually decreased in *C. gigas* during summer of 1990. It seems unlikely that this decrease in prevalence was the result of mortality of susceptible individuals. Prevalence declined from almost 90% to below 10% between late August and early November, but mortality in *C. gigas* was only about 30% during this period and, as discussed below, this mortality was not disease related. In any case, the mortality in *C. gigas* was not enough to account for the decline in prevalence. In addition, only light infections were found in *C. gigas*. Mortality in *C. virginica* is associated only with moderate and heavy *P. marinus* infections (Andrews 1988). Two possible explanations for the de-

crease in prevalence in *C. gigas* are that the parasite is able to enter *C. gigas* but is unable to proliferate or that *C. gigas* possesses an active defense mechanism that is able to eliminate the parasite. It has been shown, for example (Fisher 1988), that hemocytes from non-susceptible *C. gigas* are able to bind more *Bonamia ostreae* parasites than are hemocytes from susceptible *O. edulis*.

Heavy and moderate infections predominated in both diploid and triploid *C. virginica* and weighted incidence values approached or exceeded 4.0 for all samples during the second experiment. Weighted incidence values of 4.0 are typical of those found in heavily infected gapers (Andrews 1988) when 90% or more of the mortality is caused by *P. marinus*. On the basis of the high WI values, mortality in both diploid and triploid *C. virginica* during the second experiment can be attributed to *P. marinus*. WI values in *C. gigas* were always below 1.0 in both experiments. On the basis of previous experience with *C. virginica* and the low WI values in *C. gigas*, there did not appear to be any disease-related mortality in either diploid or triploid *C. gigas* during these experiments. The *C. gigas* mortality that did occur during the second experiment was probably not disease related; it was discovered on 7 August that the power to the pumps supplying the flume had been lost and that the flume had drained leaving the oysters exposed to air at high temperature for two days over a weekend. The highest mortality in both diploid and triploid *C. gigas* occurred soon after this event. Mortality in both *C. gigas* groups declined over the remainder of the experiment and no *C. gigas* mortality occurred after 26 September. We believe that the prolonged exposure to high temperature, not disease, caused the August *C. gigas* mortalities.

No significant differences in prevalence or intensity of *P. mari-*

*nus* infections were observed between diploid and triploid groups in oysters of the same species. However, mortality was greater in diploid *C. virginica* than in triploids after 70 days of exposure, but not in subsequent months. This early lower mortality of triploids, even though disease prevalence and intensity were similar to diploids, may suggest that triploids have a short-term energetic advantage over diploids. However, by the end of the experiment it was clear that triploidy provided no increased tolerance to *P. marinus* in *C. virginica* and the hypothesis that energy resources diverted from gametogenesis in triploid individuals can be used to enhance defense mechanisms does not seem to be true. On the basis of these experiments triploid *C. virginica* can not be expected to provide any disease resistance advantage over diploid individuals. However, the faster growth rate of triploid *C. virginica* compared to diploid *C. virginica* (Barber and Mann 1991), would result in triploids reaching marketable size sooner than diploid individuals and thus potentially incurring fewer losses to disease.

The tolerance of *C. gigas* to *P. marinus* suggests that this oyster species has potential to assist the revitalization of the Chesapeake Bay oyster industry. On the basis of these experiments there should be very little, if any, *C. gigas* mortality associated

with *P. marinus* infections. Triploid *C. gigas* appeared to be at least as disease tolerant as diploid individuals, although the *C. gigas* triploid group was only 67% triploid at the beginning of the experiment and only 36% triploid when it was terminated. We feel that this decline in triploidy was probably a result of sampling error rather than differential mortality between diploid and triploid individuals, but this could not be determined with certainty. On the basis of these experiments, the use of *C. gigas* as part of a revitalization program would offer the advantage of disease tolerance in areas where *P. marinus* is endemic. In addition, use of triploid *C. gigas* would also reduce the risk of undesirable introductions.

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## DISEASE INCIDENCE AND POTENTIAL MECHANISMS OF DEFENSE FOR MSX-RESISTANT AND -SUSCEPTIBLE EASTERN OYSTERS HELD IN CHESAPEAKE BAY<sup>1</sup>

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**ABSTRACT** A disease of eastern oysters, *Crassostrea virginica* (Gmelin, 1791), caused by a protistan, *Haplosporidium nelsoni*, has caused great losses in the oyster fisheries of the northern Atlantic coast of North America. Certain oyster stocks have been selectively bred to survive infection by *H. nelsoni* (often called MSX disease) but mechanisms of resistance are not known and potential resistance to another protistan parasite, *Perkinsus marinus*, is not clear. Oysters from MSX-resistant stocks and from unselected (susceptible) stocks were compared over one year (1988-89) at an estuarine site in Chesapeake Bay where MSX disease and *P. marinus* ("dermo" disease) are both known to occur. Prevalence of MSX disease was 0%-4% for the resistant stock and 36%-60% for the susceptible stock, whereas prevalence of *P. marinus* was similar for both resistant (58%) and susceptible (67%) stocks. Comparison of putative defense mechanisms revealed no differences in hemocyte capacity to spread, respond to salinity changes or locomote *in vitro*. The susceptible stock exhibited higher serum protein concentrations and higher lysozyme concentrations during spring and summer. Serum agglutination titers for *Vibrio cholerae* CA401 were consistently higher for the resistant stock during summer; serum lectins could be related to disease resistance or affected by *H. nelsoni* infection.

**KEY WORDS:** eastern oyster, *Crassostrea virginica*, *Haplosporidium nelsoni*, *Perkinsus marinus*, invertebrate immunology, defense mechanisms, lectins

### INTRODUCTION

Eastern oysters, *Crassostrea virginica*, are subjected to two common parasites in the Chesapeake Bay, *Haplosporidium nelsoni* (Haskin, Stauber, Mackin) (MSX disease) and *Perkinsus marinus* (Mackin, Owen, Collier) ("dermo" disease). In the 1950's, *P. marinus* was the primary cause of oyster deaths in the lower Chesapeake Bay, with most infections caused by direct transmission from dying oysters (Andrews 1979, 1988, Andrews and Ray 1988). A recent decline in abundance of *P. marinus* may be a consequence of depleted oyster populations in the lower Chesapeake Bay, but the disease still persists at the fringes of its salinity tolerance (12-15 ppt) where natural recruitment of oysters occurs (Andrews 1979, Andrews and Ray 1988).

Following the discovery of MSX in 1957 in Delaware Bay, this protozoan has caused heavy mortalities of *C. virginica* (up to 95%) (Ford and Haskin 1988). Chronic disease pressure with occasional epizootic outbreaks continues to suppress eastern oyster production in the Chesapeake and Delaware Bays (Ford and Haskin 1988). After 1960, MSX disease spread throughout lower Chesapeake Bay with annual mortality rates of 50-60% and replaced *P. marinus* as the major cause of oyster mortalities (Andrews 1979). The disease caused by MSX parasites tends to exhibit uniform levels of infection and kills oysters over wide areas within a 15-25 ppt salinity range (Andrews 1979).

Researchers at Rutgers University have bred, in the laboratory, survivors of MSX-exposed oysters from Delaware Bay to produce MSX-resistant stocks (Haskin and Ford 1979, Ford and Haskin

1987). "Resistance" in this case does not prevent infection but enables host oysters to restrict the parasites to small, non-lethal lesions in gill or palp epithelia (Ford and Haskin 1987, 1988). The exact mechanism for this resistance is unknown. Valiulis (1973, also see Ford 1988 and Ford and Haskin 1987) demonstrated that selection for survival against *H. nelsoni* might also improve survival against *P. marinus* at low dosage. However, Burreson et al. (1990) exposed MSX-resistant oysters to the natural conditions in the lower Chesapeake Bay for two years, resulting in 96% prevalence of *P. marinus* and 45% mortality.

Hemocytes of marine bivalves, by phagocytosis, make a major contribution to host defense against pathogens and parasites (Foley and Cheng 1975). This requires that hemocytes be able to recognize, bind, ingest and dispose of exogenous or nonself particles (Fisher 1988a). In addition, hemocytes are believed to release lysosomal enzymes into the hemolymph to destroy bacteria or alter foreign particles so they can be recognized as nonself (Foley and Cheng 1977). It has been hypothesized that lectins (non-immunoglobulin proteins) in invertebrate serum can enhance the phagocytic response by marking, or "recognizing" nonself material (Olafsen 1988).

Occurrence and intensity of *H. nelsoni* and *P. marinus* infections were monitored in two eastern oyster stocks. Presumptive defense components in MSX disease resistance were monitored in both resistant and susceptible stocks held at the same site in Chesapeake Bay over a one-year period. Hemocyte spreading, salinity regulation and locomotion, and serum levels of protein, lysozyme and agglutinin (vs. *Vibrio cholerae*) were assayed.

### MATERIALS AND METHODS

Two stocks of oysters were maintained at Deal Island, Maryland from June 1988 to July 1989. An MSX-resistant, Delaware Bay stock (CXF) was selected over seven generations at the

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Harold Haskin Research Laboratory (HHRL) for resistance to mortality from MSX disease (Dr. Susan Ford, pers. comm., Haskin and Ford 1979). The resistant stock was spawned at HHRL in July 1986 and progeny were maintained in trays in Delaware Bay at Cape Shore, New Jersey until transport to Maryland. The susceptible stock was dredged from the Tred Avon River in the Maryland portion of the Chesapeake Bay in June 1988. Initial oyster sizes for both stocks ranged from 40–80 cm length (mean for both groups was 60 cm). All oysters were placed in trays and suspended in 2 m of water, approximately 1/2 m from the bottom at the Maryland Department of Natural Resources and University of Maryland Oyster Hatchery at Deal Island, MD. Three trays held 180 resistant stock each and three trays held 100 susceptible stock each. Salinity and temperature ranged from 18 ppt and 30°C in the summer to 12 ppt and -1°C in the winter. Oysters were monitored for survival weekly in spring and summer and monthly in fall and winter. Samples were collected for disease diagnosis, hemocyte assays and serum measurements in June 1988, November 1988, May 1989 and July 1989. All data were analyzed by Student's t-test for paired comparisons (Sokal and Rohlf 1981) on Statistical Analysis Systems (SAS).

#### Disease Diagnosis

Infection of oysters by *P. marinus* was determined by incubating a 1 cm<sup>2</sup> section of rectal tissue in thioglycollate-estuarine water (15 ppt) medium for 7 d as described by Ray (1966). Intensity of infection was determined by examination of hyphospores and rated according to Ray (1954): 0—negative, 1—light, 2—light/moderate, 3—moderate, 4—moderate/heavy and 5—heavy. The weighted incidence of dermo disease (WI) was calculated as described by Andrews (1988) and is a combination of the prevalence and intensity of infections.

Incidence of *H. nelsoni* was diagnosed as in Ford (1985) with intensity scored as follows: 0—negative, 1—gill/epithelial infections, 2—subepithelial/local, 3—light systemic and 4—advanced systemic.

#### Hemocyte Assays

Twelve oysters from each stock were collected and their shells notched at the posterior-ventral edge to allow withdrawal of 0.5 mL hemolymph from the adductor muscle by syringe. Hemocyte activity assays (hemocyte spreading, volume regulation and locomotion) were conducted as described by Fisher and Newell (1986) and Fisher et al. (1989).

#### Serum Assays

A second 0.5 mL sample of hemolymph was withdrawn from each oyster and centrifuged (300 × g) to remove hemocytes. Serum was refrigerated and retained for analysis of protein and lysozyme content and determination of agglutination titer (performed within two days of sampling).

Protein concentration of cell-free serum was determined by the Bio-Rad<sup>1</sup> protein assay using 10 µL serum measured on a Shimadzu<sup>1</sup> spectrophotometer at 595 nm with a bovine plasma gamma globulin standard. Serum lysozyme concentration was determined using a standard suspension (15 mg/100 mL) of *Micro-*

*coccus lysodeikticus* (Sigma<sup>1</sup>) by the method of Shugar (1952) as described in Chu and La Peyre (1989). A sample of serum (0.2 mL) was monitored for degradation of lysozyme for 3 min at room temperature (21°C) at 450 nm on a Cary spectrophotometer and compared to hen egg-white standards.

Agglutination of *Vibrio cholerae* CA401 was tested in round-bottom microtiter plates after serial twofold dilution of cell-free serum with ASW at 18 ppt (Tamplin and Fisher 1989). Bacteria concentrations were adjusted to  $\sim 3 \times 10^8$  (using standard McFarland nephelometer units) and added 1:1 to the diluted serum. Bacteria were allowed to settle for 16–24 h before reading. Titer was recorded as the reciprocal of the highest dilution where agglutination was observed. Averages and statistics were compiled with log<sub>2</sub> titer values.

## RESULTS

#### Disease Diagnosis

The MSX-resistant oyster stock remained relatively uninfected by *H. nelsoni* in the year they were maintained at Deal Island (only 1 animal of 75 tested positive for MSX; Table 1). The susceptible stock however, showed initial infections in 36% of the oysters with an average intensity of 1.8 (ranging from 1–3). This grew to 60% infections by November when intensity averaged 3.0 (ranging from 1–4). By July 1989, nearly all susceptible animals were dead.

Stocks were not tested for infection with *P. marinus* at the time of planting (June 1988) at Deal Island. At that time, *P. marinus* was believed absent from Delaware Bay, so the resistant stock was presumed uninfected. Eight percent of resistant oysters were infected with *P. marinus* disease in November 1988, but that rose to 58% by July 1989 (Table 1). The weighted incidence (WI) of infection also increased from 0.1 in November 1988 to 1.8 by July 1989. There were eight gapers (dead oysters) from the resistant stock in July 1989 and all were infected with *P. marinus* with a WI of 4.0 (±0.5). The initial prevalence of *P. marinus* for the susceptible stock is unknown, but 20% of the oysters were infected in November 1988 and May 1989 and 67% were infected by July 1989 (Table 1). The WI for infection of susceptible oysters was less than 0.5 for the first two collections but increased to 1.4 by July 1989.

#### Hemocyte Assays

There were no significant differences at the  $p < 0.05$  level between the two oyster stocks in time to hemocyte spreading at

TABLE 1.

Prevalence and weighted incidence (WI) of *P. marinus* (dermo) and *H. nelsoni* (MSX) infections for MSX-resistant and MSX-susceptible oyster stocks.

Date	N	Resistant			Susceptible		
		% MSX	% Dermo	WI	% MSX	% Dermo	WI
Jul '88	25	0	nd	nd	36	nd	nd
Nov '88	25	4	8	0.1	60	20	0.4
May '89	25	0	28	0.4	nd	20	0.2
Jul '89	15	nd <sup>1</sup>	58	1.8	nd	67	1.4

<sup>1</sup> Eight gapers from the resistant stock were tested in July 1989 and 100% were infected by *P. marinus* with a WI = 4.0 rating. nd = no data.

<sup>1</sup> Mention of commercial products or companies does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

ambient salinity (18 ppt) on any of the collection dates (Fig. 1). Nor were there significant differences in salinity regulation by the hemocytes with the single exception of faster adjustment to hyperosmotic conditions (36 ppt salinity) by hemocytes of resistant oysters in June 1988 (Fig. 1). Likewise, there were no significant differences at the  $p < 0.05$  level between the two stocks in average rate of locomotion (Fig. 2).

**Serum Assays**

Protein concentration in cell-free hemolymph tended to be higher in the susceptible oysters (Fig. 3), but this difference was significant ( $p < 0.05$ ) only in the May 1989 sample. Both stocks had higher serum protein levels in the winter than in spring and summer (Fig. 3).

Lysozyme levels did not show the same seasonal pattern as protein. In spring and summer, the susceptible stock exhibited higher concentrations of serum lysozyme (Fig. 4), but this was significant ( $p < 0.05$ ) only in July 1989.

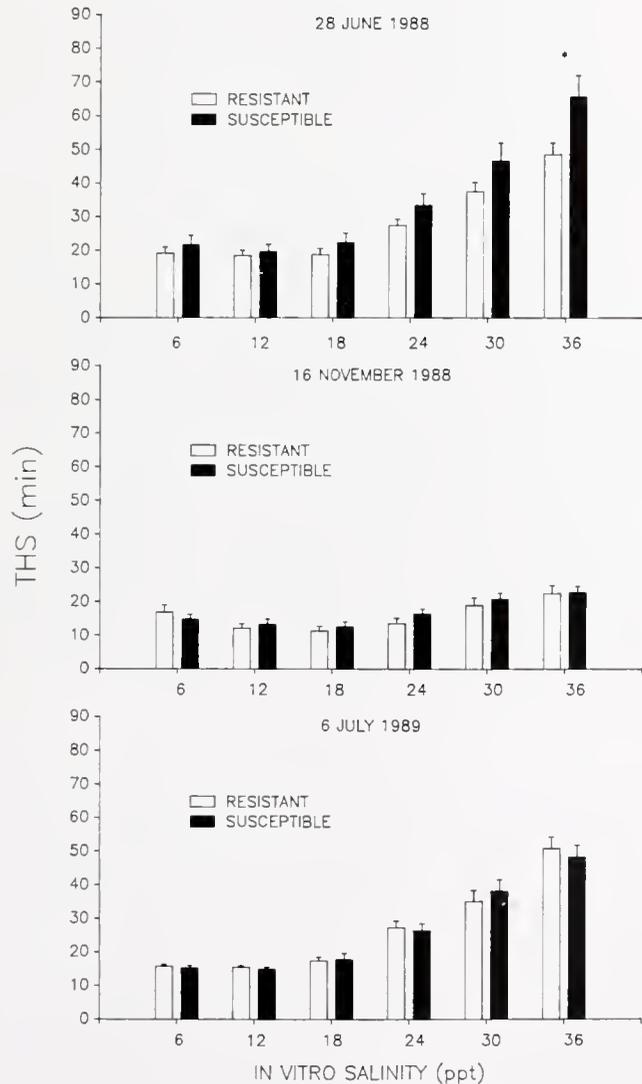


Figure 1. Average time to hemocyte spreading (THS) at 15°C and six *in vitro* salinities for MSX-resistant and -susceptible oysters held at Deal Island from 1988–1989. Error bars are +1 SE and n = 12 for each bar. \*marks significance at  $p < 0.05$  level.

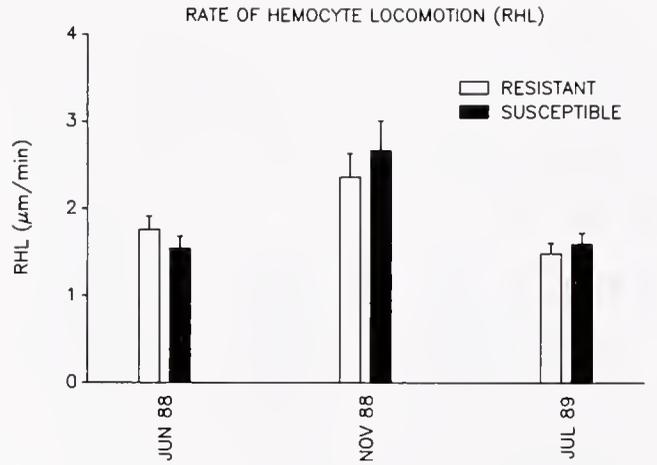


Figure 2. Average rate of hemocyte locomotion (RHL) for MSX-resistant and -susceptible oysters held at Deal Island from 1988–1989. Locomotion assays were conducted at 15°C and ambient salinity (18 ppt) within 90 mins of hemocyte spreading. Error bars are +1 SE and n = 12–15 for each bar.

Titers for agglutination of *V. cholerae* CA401 by cell-free serum demonstrated a continuous and significant decline from July 1988 to July 1989 (Fig. 5). This decline was more pronounced in the resistant stock. Titers were consistently higher in the resistant stock and the difference was statistically significant ( $p < 0.05$ ) for the June 1988, July 1988 and July 1989 samples. A similar result was obtained for a single sampling of different resistant and susceptible stocks in October 1988 at HHRL (Tamplin and Fisher 1989). In this sample, the MSX-resistant stock had a higher mean titer ( $4.3 \pm 0.8$ ) than the susceptible stock ( $2.8 \pm 0.5$ ) but the difference was not significant at the  $p < 0.05$  level.

**DISCUSSION**

Selected, MSX-resistant, Delaware Bay oysters held in Chesapeake Bay at an intermediate salinity (12–18 ppt) were found to resist infection by *H. nelsoni* whereas unselected and susceptible Chesapeake Bay oysters held at the same site became increasingly infected. The results imply that the MSX-selected oysters were

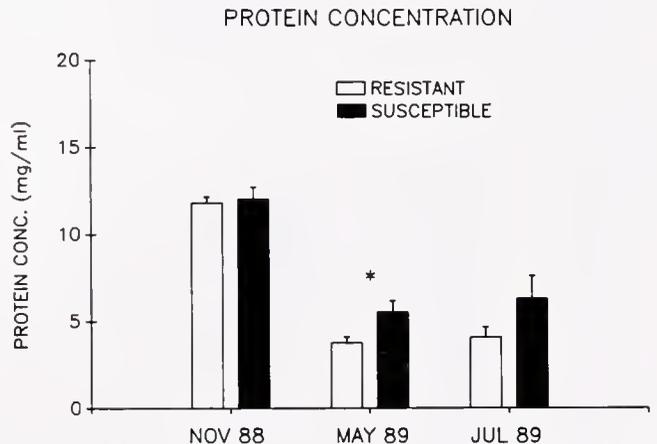


Figure 3. Mean hemolymph protein concentration (mg/mL) for MSX-resistant and -susceptible oysters held at Deal Island from 1988–1989. Error bars are +1 SE and n = 25 for each bar. \*denotes significance at  $p < 0.05$  level.

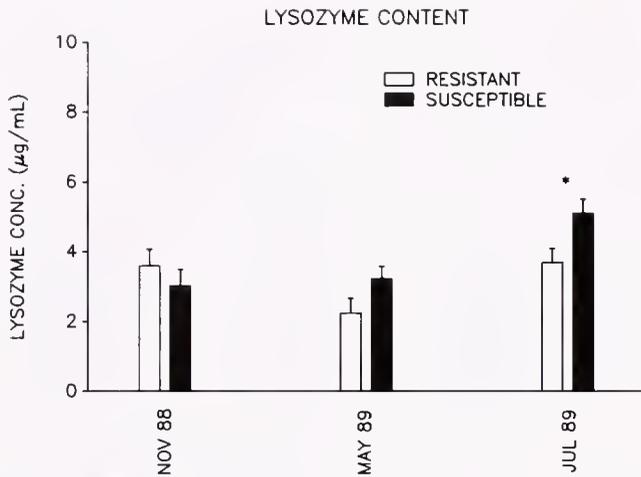


Figure 4. Mean lysozyme concentration ( $\mu\text{g/mL}$ ) in hemolymph from 25 MSX-resistant and -susceptible oysters held at Deal Island from 1988-1989. Error bars are  $\pm 1$  SE and \* denotes significance at  $p < 0.05$  level.

resistant not only to *H. nelsoni*-related mortality as indicated by previous studies (Haskin and Ford 1979, Ford and Haskin 1987, 1988, Ford 1988), but also to infection since only 1% of all resistant oysters tested positive for MSX. Both stocks were equally susceptible to *P. marinus* infection, supporting the conclusions of Bureson et al. (1990) that MSX resistance may be due to a relatively specific factor that does not confer resistance to dermo disease.

Hemocyte activities (spreading, salinity regulation and locomotion) from oysters of both stocks exhibited the same seasonal pattern of decreased capacity during the "summer stress" period (Figs. 1 & 2) as described in Fisher et al. (1989). Since there were no differences between resistant and susceptible stocks however, these hemocyte activities do not appear to be directly involved in resistance to *H. nelsoni*. Ford (1988) suggested that phagocytosis by hemocytes may not be a particularly active defense against

MSX disease and noted that only the less phagocytic agranular hemocytes appear at sites of *H. nelsoni* infection.

Serum lysozyme, believed to be synthesized in the hemocytes and released via degranulation (Foley and Cheng 1977, Cheng et al. 1975), was significantly higher in the susceptible stock in July 1989 (Fig. 4). This difference could be related to the 60% *H. nelsoni* rate of infection at that time. The role of serum lysozyme is poorly understood in relation to parasite burden and is further obscured by annual variations in concentration (Feng and Cannonier 1970, Cheng et al. 1975, Chu and La Peyre 1989).

The only consistent difference in hemolymph components between stocks was higher agglutination titers of resistant oysters to *Vibrio cholerae* CA401 (Fig. 5). Tamplin and Fisher (1989) found a similar, but not statistically significant, situation with two other resistant (WJDX) and susceptible (VAJP) oyster stocks. Ling (1990) also found that MSX-resistant oysters had higher agglutinating titers for latex beads. However, he also found that agglutinating ability in MSX-susceptible oysters decreased with increased MSX infection levels.

Agglutination by invertebrate sera is usually caused by lectins (multivalent, nonimmunoglobulin proteins) which may serve a wide variety of recognition functions (Sharon and Lis 1972). In invertebrates lectins may act as opsonins that recognize or label nonself material to increase the phagocytic response (Vasta et al. 1982, Renwartz and Stahmer 1983, Olafsen 1988). Eastern oyster serum was recently shown to agglutinate a broad range of marine bacteria with high specificity and varying intensity (Fisher and DiNuzzo 1990). Fisher (1988b) suggested that better recognition of the parasite *Bonamia ostreae* by hemocytes was a factor in the insusceptibility of *Crassostrea gigas* relative to *Ostrea edulis*. The fact that eastern oyster hemocytes can readily phagocytose dead, but not live, MSX parasites (Ford et al. 1990) may be indirect evidence that the ability to recognize this parasite as nonself is an important mechanism of defense. Thus, it is possible that higher titers of serum lectins can help resistant oysters identify parasites as nonself and heighten the defense response.

Two potential mechanisms of MSX resistance, hemocyte capacity and serum lysozyme activity, were not supported by the results of this study. The role of serum lectins was not elucidated, but there was a consistent difference in lectin titers between MSX-resistant and -susceptible oysters. It is not clear whether higher agglutination titers against *V. cholerae* CA401 in the resistant stock is associated directly or indirectly with improved survival or is merely an effect of MSX and/or dermo infections.

#### ACKNOWLEDGMENTS

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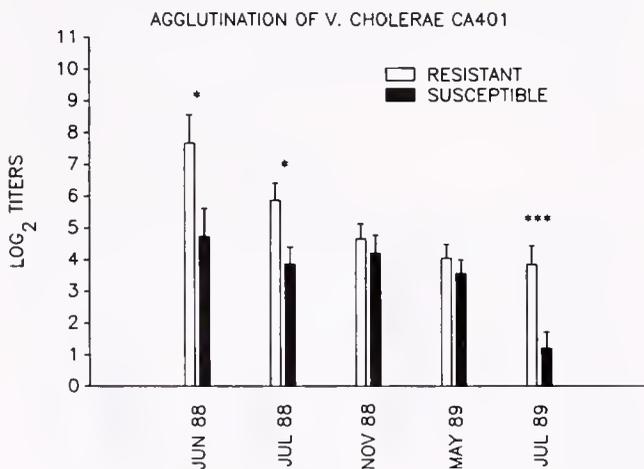


Figure 5. Average  $\log_2$  titer of agglutination of *Vibrio cholerae* CA401 by hemolymph of MSX-resistant and -susceptible oysters held at Deal Island from 1988-1989. Error bars are  $\pm 1$  SE and \* denotes significance at  $p < 0.05$  level and \*\*\* denotes significance at  $p < 0.001$  level.

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## STERILE TRIPLOID *CRASSOSTREA VIRGINICA* (GMELIN, 1791) GROW FASTER THAN DIPLOIDS BUT ARE EQUALLY SUSCEPTIBLE TO *PERKINSUS MARINUS*<sup>1</sup>

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**ABSTRACT** Growth, tolerance of *Perkinsus marinus*, and gametogenesis of diploid and triploid Eastern oysters, *Crassostrea virginica* (Gmelin, 1791) were compared in the York River, Virginia between June 1989 and November 1990. Triploid oysters had significantly greater mean shell height ( $P \leq 0.02$ ) and whole weight ( $P \leq 0.005$ ) than diploid oysters throughout the study period. In November 1990, triploids had significantly greater mean dry tissue weight ( $P \leq 0.006$ ) than diploids. On average, triploid oysters reached commercial size (63.5 mm) 5 months before diploid oysters. Diploid and triploid groups became similarly infected with *P. marinus* during summer 1990. Prevalences reached 96% with moderate and heavy intensities in both groups in October 1990. Gonadal development in triploids was significantly reduced ( $P \leq 0.02$ ) compared to development in diploids. While diploid oysters completed a normal gametogenic cycle, gametogenesis in triploid male oysters was arrested prior to gamete maturation in all but one individual, and in triploid females only a few isolated oocytes were produced. Potential commercial utilization of triploid *C. virginica* will most likely take advantage of superior growth rates compared to diploids, as disease tolerance was not improved.

**KEY WORDS:** oysters, triploid, growth, gametogenesis, disease, *Crassostrea virginica*, *Perkinsus marinus*

### INTRODUCTION

Triploid oysters (having three sets of chromosomes) represent an increasing proportion of hatchery oyster (*Crassostrea gigas* Thunberg) production in the U.S. Pacific Northwest (Oregon and Washington), which now accounts for approximately 37% of total U.S. production (U.S. National Marine Fisheries Service, 1990 Landings for the United States). The commercial value of triploid oysters results from the physiological alteration that occurs as the result of having an extra set of chromosomes. Most notably, triploid *C. gigas* exhibit retarded gonadal development compared to diploid (normal) oysters (Allen and Downing 1986, 1990). Thus being relieved of the metabolic requirements of gametogenesis, triploid oysters exhibit greater somatic growth and a more consistent glycogen content than diploid oysters, making them a better market product throughout the year (Allen and Downing 1986, Davis 1989). Triploid *C. gigas* are also less susceptible to "summer mortality," which has been related to stress induced by excessive gonadal maturation (Perdue et al. 1981, Beattie et al. 1988).

As reviewed by Haskin and Andrews (1988) and Andrews (1988), native stocks of *Crassostrea virginica* Gmelin along the east coast of the United States have been severely depleted by two oyster pathogens, *Haplosporidium nelsoni* and *Perkinsus marinus*. At sublethal levels, the diseases caused by these pathogens inhibit growth, reduce fecundity, and lower condition and glycogen content (Menzel and Hopkins 1955, Newell 1985, Barber et al. 1988a, 1988b, Crosby and Roberts 1990, Paynter and Bureson 1991). Although resistance to mortality caused by *H. nelsoni* has been achieved through selective breeding (Ford and Haskin 1987), no increase in tolerance to *P. marinus* has been effected to date. Thus the impact of *P. marinus* continues unabated.

Evaluation of the performance of triploid *C. virginica* with respect to growth, disease tolerance, and gametogenesis is limited. Compared to their diploid counterparts, triploid *C. virginica* have

been reported to grow faster (Stanley et al. 1984), exhibit inhibited gametogenesis (Lee 1988), and have similar susceptibility to *P. marinus* in dosed flumes (Meyers et al. 1991). This study provides the first coordinated examination of growth, disease tolerance, and gametogenesis of triploid *C. virginica* in the field, so a preliminary evaluation of the potential value of triploid oysters to the aquaculture industry of the eastern U.S. can be made.

### MATERIALS AND METHODS

Broodstock oysters obtained from Mobjack Bay, Virginia, were conditioned in the Virginia Institute of Marine Science (VIMS) hatchery in early 1989 at 20–24°C with a diet consisting of *Isochrysis galbana* (Tahitian), *Thalassiosira pseudonana* (3H), *T. weissflogii*, and *Chaetoceros calcitrans*. Spawning was induced by elevating the temperature to 28–30°C and adding a sperm suspension, if necessary. Triploid larvae were produced from oysters (3♀ and 2♂) spawned on 23 March, 1989, and diploid larvae were produced from oysters (6♀ and 8♂) spawned on 5 April, 1989. Triploidy was induced by adding (15 min after fertilization) 1.0 mg cytochalasin B (CB) in 1.0 ml DMSO to a 2 l beaker containing embryos in 1 l of seawater. Treatment with CB lasted 15 min and was followed by a 15 min DMSO (1.0 ml/l) exposure (Downing and Allen 1987, Allen et al. 1989).

Larvae were reared in 400 gal conical tanks. Water was changed every 1–2 days and food (*I. galbana*, *T. pseudonana*, *T. weissflogii*, and *C. calcitrans*) was added 1–2 times daily. Eyed larvae were set onto "minicultch" (ground oyster shell) and placed into upwellers receiving raw water from the York River, Virginia. Oysters were removed from the upwellers in July and placed into trays that were hung off a pier at the VIMS campus (York River, Virginia). Twenty-five individuals from each group were assayed for ploidy by Dr. S. Allen, Rutgers University, using flow cytometry (Chaiton and Allen 1985). The CB-treated group contained 96% triploids, while the untreated group contained 0% triploids.

Regular determinations of shell height (maximum dimension from hinge to opposite margin) and whole (live) weight, began in June and August 1989, respectively, and were continued until

<sup>1</sup>Contribution No. 1713 from the Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

November 1990. The number of individuals measured was 30 per group prior to November 1989 and 50 per group from November 1989 onward. Additionally, in November 1990, 60 oysters from each group were shucked for determination of dry tissue weight (80°C).

Beginning in August 1989, 25 individuals from each group were sacrificed periodically for determination of prevalence and intensity of *Perkinsus marinus*, using the fluid thioglycollate method (Ray 1952). Prevalence was the percentage of oysters found to have infections, and the intensity of infection in each oyster was rated as light (L), moderate (M), or heavy (H) (Ray 1954).

Gametogenesis was monitored from April through October 1990. Twenty individuals from each group were sacrificed and fixed in Davidson's AFA. A standard transverse (anterior) section of the visceral mass taken beginning at the level of the intersection of the labial palps and gills (and including gill, mantle, stomach, intestine, and digestive diverticula) was then dehydrated, cleared, and embedded in Paraplast. Six- $\mu$ m sections were mounted on slides and stained with Harris' Hematoxylin and Eosin Y. To quantify gonad development, a gonadal area index (GAI) was determined from the histological section of individuals found to be sexually differentiated, as the ratio of [gonadal area/area of entire visceral mass]  $\times$  100. GAI represents the proportion of total cross sectional area that is comprised of gonadal tissue and is indicative of gonadal development (gametogenesis) and spawning (Barber et al. 1988a, 1991).

Comparisons of mean shell height, whole weight, dry tissue weight (November 1990 only), and GAI (after arcsin transformation) between diploid and triploid groups were made for each sampling date using a t-test.

## RESULTS

### Growth

Growth of all oysters (measured both as shell height and whole weight) occurred primarily in fall (September–December) 1989 and 1990 and spring (April–July) 1990 but slowed considerably during winter (December–April) 1989–90 and summer (July–October) 1990 (Figures 1 and 2).

Mean shell height of diploid oysters increased from 10.9 mm in June 1989 to 64.6 mm in November 1990, while mean shell height

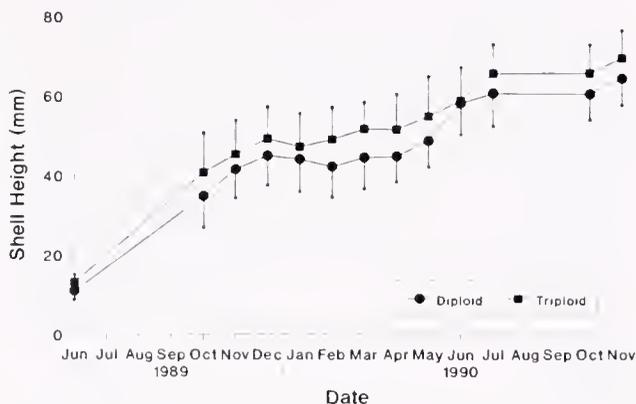


Figure 1. Mean shell height (mm) of diploid and triploid oysters, *C. virginica*, grown in the York River, Virginia, between June 1989 and November 1990. Error bars represent 1 SD.



Figure 2. Mean whole weight (g) of diploid and triploid oysters, *C. virginica*, grown in the York River, Virginia, between August 1989 and November 1990. Error bars represent 1 SD.

of triploid oysters increased from 13.1 mm to 69.7 mm over the same time interval (Figure 1). Means of the triploid group were significantly greater ( $P \leq 0.02$ ) than means of the diploid group on all sampling dates except January and June 1990. From October 1989 through November 1990, triploid oysters maintained an average 5 mm shell height differential over diploid oysters. A mean shell height of 63.5 mm (considered commercial size) was attained by triploid oysters in July 1990 and by diploid oysters in November 1990 (Figure 1). In July 1990, 66% of the triploid group exceeded 63.5 mm in shell height compared to 38% of the diploid group.

Between August 1989 and November 1990, mean whole weight of diploid oysters increased from 1.6 g to 40.6 g, and mean whole weight of triploid oysters increased from 3.4 g to 52.6 g (Figure 2). Means of the triploid group were significantly greater ( $P \leq 0.005$ ) than means of the diploid group at all sampling dates except June 1990. Over the course of the study, the differential in whole weight between triploid and diploid oysters increased. In August 1989, the difference between mean whole weight of diploid and triploid oysters was 1.8 g, but by October 1990 this difference had increased to 13.8 g (Figure 2).

In November 1990, mean dry tissue weight of triploid oysters was 1.1 g compared to 0.9 g for diploid oysters. These means were significantly different ( $P \leq 0.006$ ).

### Disease

*P. marinus* was not detected in diploid or triploid oysters (prevalence = 0%) in either August or September 1989, shortly after being placed into the York River, VA (Table 1). In October and again in November 1989, 1–2 individuals in each group had extremely light infections (1–2 *P. marinus* cells). In April 1990, prevalence was reduced to 0%. In July 1990, prevalence of *P. marinus* was 56% in the diploid group and 44% in the triploid group. Most infections were light, but there were also 3 moderate infections and 1 heavy infection in each group. By October 1990, prevalence had increased to 96% in both groups, with a concomitant increase in both moderate and heavy infections. Although no accurate counts were obtained, mortalities were observed in both diploid and triploid groups beginning in July 1990.

### Gametogenesis

Mean GAI of both diploid and triploid oysters was 0% in April 1990 (Figure 3). Beginning in May, mean GAI of diploid oysters

TABLE 1.

Prevalence (%) and intensity (L = light; M = moderate; H = heavy) of *P. marinus* infections in diploid and triploid oysters, *C. virginica* from August 1989 to October 1990; n = 25.

Date	Group	Prevalence (%)	Intensity L-M-H
14 August 1989	Diploid	0	0-0-0
	Triploid	0	0-0-0
13 September 1989	Diploid	0	0-0-0
	Triploid	0	0-0-0
16 October 1989	Diploid	4	1-0-0 <sup>1</sup>
	Triploid	8	2-0-0 <sup>1</sup>
13 November 1989	Diploid	4	1-0-0 <sup>1</sup>
	Triploid	4	1-0-0 <sup>1</sup>
16 April 1990	Diploid	0	0-0-0
	Triploid	0	0-0-0
31 July 1990	Diploid	56	10-3-1
	Triploid	44	7-3-1
11 October 1990	Diploid	96	15-5-5
	Triploid	96	16-2-6

<sup>1</sup> These infections were based on the detection of only 1 or 2 *P. marinus* cells in the thioglycollate culture.

increased to values of 18.3% in June, 17.8% in July, and 16.8% in August. Mean GAI for the diploid group decreased to 10.5% in October. Mean GAI of triploid oysters never exceeded 8%. Mean GAI values for diploid and triploid groups were significantly different ( $P \leq 0.02$ ) for all sampling dates after April 1990 (Figure 3), indicating that triploid oysters had considerably lower gonadal production than diploid oysters.

Diploid oysters were observed to undergo a typical gametogenic cycle. All individuals were undifferentiated in April (Table 2). By May gametogenesis was initiated, as 8 of 20 individuals were differentiated. In June, July, and August, 57 of 60 individuals were differentiated and contained oocytes and spermatocytes, as well as mature gametes (Figures 4A, 4B). In the October sample, it was obvious that spawning had occurred in 17 of the 20 individuals examined, as follicles were reduced in size and at least partially devoid of mature gametes. In a few cases, spawning and resorption were so complete that sex could no longer be determined (Table 2).

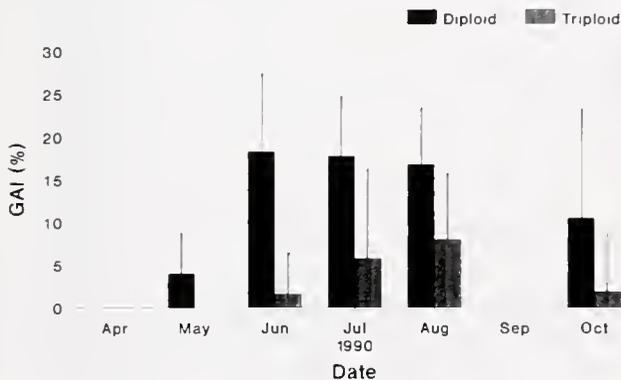


Figure 3. Mean gonadal area index (GAI, %) of diploid and triploid oysters, *C. virginica*, grown in the York River, Virginia, between May and October 1990. Error bars represent 1 SD.

TABLE 2.

Number of undifferentiated (U), male (M), and female (F) diploid and triploid oysters, *C. virginica*, from April–October 1990 and notes on gametogenesis; n = 20.

Date	Diploid Group U-M-F	Triploid Group U-M-F
16 April	20-0-0	20-0-0
21 May	12-7-1	20-0-0
20 June	2-11-7 (sperm; ova)	14-1-5 (1 had sperm) <sup>1,2</sup>
24 July	0-12-8 (sperm; ova)	7-6-7 (1 had sperm) <sup>2</sup>
23 August	1-15-4 (sperm; ova)	4-13-3 (1 had sperm; 1 had ova) <sup>2</sup>
11 October	6-9-5 (17 spawned)	16-3-1 (1 had ova; <sup>2</sup> 0 spawned)

<sup>1</sup> This individual was a hermaphrodite.

<sup>2</sup> This individual was later identified as a diploid.

Triploid oysters did not undergo a normal gametogenic cycle. In both April and May, all individuals were undifferentiated (Table 2). In June, July, and August, the number of differentiated individuals increased from 6 to 13 to 16, respectively. Females

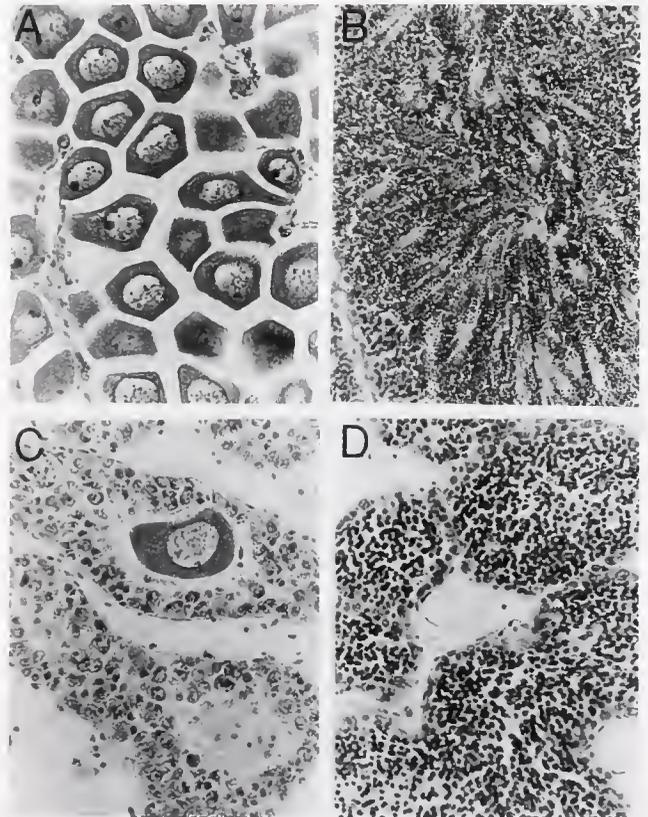


Figure 4. Gonadal sections of: (A) Mature diploid female with oocytes. (B) Mature diploid male with spermatocytes and spermatozoa. (C) Typical triploid female with single, isolated oocyte. (D) Typical triploid male with spermatocytes but no spermatozoa. Magnification of all plates is 400 $\times$ .

were distinguished by the presence of very few oocytes, most often isolated in separate follicles containing no other developing oocytes (Figure 4C), and males typically contained spermatocytes but no spermatozoa (Figure 4D). In October, the number of undifferentiated individuals increased to 16 (Table 2). There was no evidence of spawning (follicles partially devoid of gametes or gametes in gonoducts) in any of the triploid oysters. Instead, infiltration of follicles by phagocytes was evident, indicating resorption of gametes.

Of the 120 oysters examined from the 96% triploid group, mature gonads were found in only five individuals, one of which was a hermaphrodite (Table 2). Samples of adductor muscle tissue from these individuals were subsequently assayed for ploidy (S. K. Allen, Rutgers University), and 4 of the five were diploid, the exception being a male from the 23 August sample.

## DISCUSSION

### Growth

Triploid oysters were significantly larger than diploid oysters, both in terms of shell height and whole weight, throughout the period of this study, which ended when oysters were about 1.5 yrs of age. The 2 week age difference between diploid and triploid groups, although potentially important initially, was not considered a factor after the age of 1 yr. A difference in shell height of about 5 mm was maintained between triploids and diploids throughout most of the study period. In the case of whole weight, which includes both shell and tissue components, the difference between triploids and diploids appeared to increase over time, suggesting that tissue production was greater in triploids. This was verified by the significantly greater dry tissue weight found in triploid oysters than in diploid oysters in November 1990.

Growth rate was generally greater during fall and spring than during winter and summer. Oyster growth is generally reduced during winter as the result of lower temperatures and decreased clearance rates. The decrease in growth rate during summer 1990 occurred in conjunction with infection by the oyster pathogen *P. marinus*, which has been shown to negatively affect oyster (shell) growth (Menzel and Hopkins 1955, Paynter and Bureson 1991). Gametogenesis also occurs in summer, and is known to retard shell growth in oysters (Mann 1979, Allen and Downing 1986). Because both diploid and triploid groups experienced reduced growth in summer 1990, disease, rather than differential gonadal development, is the most likely cause.

In general, because triploid oysters invest less energy in the production of gametes, more energy is available for somatic growth. Allen and Downing (1986) found that triploid *C. gigas* continue to grow and use less of their stored glycogen than diploids during the normal gametogenic period, when growth in diploids ceases. Growth differences between diploid and triploid *C. gigas* may depend upon environmental conditions and resulting glycogen utilization patterns (Davis 1988, 1989). Triploid scallops, *Argopecten irradians* and *Chlamys nobilis*, displayed greater somatic growth compared to diploid scallops as a result of retarded gonadal development and reduced glycogen utilization (Tabarini 1984, Komaru and Wada 1989). Superior growth of triploid organisms may also be associated with the increased heterozygosity resulting from the extra set of chromosomes. Stanley et al. (1984) reported that triploid *C. virginica* grew faster and were more heterozygous than diploid siblings only if triploidy were accomplished by blocking meiosis I. Triploid *Mya arenaria* were also

found to be nearly twice as heterozygous as their diploid siblings (Mason et al. 1988).

### Disease

The results of this study indicate that triploid *C. virginica* are no more tolerant of *P. marinus* than diploid oysters. Because oysters were placed in the field late in 1989, infections incurred that year were rare and extremely light. These infections were apparently lost (or became subclinical) over winter, as *P. marinus* was undetected in April 1990. By July, however, about 50% of both diploid and triploid groups were infected, and this increased to 96% by October, with similar intensities and mortalities in both groups. Thus triploid *C. virginica* become infected with *P. marinus* in nature to the same extent as diploids in spite of their presumed energetic advantage. Similar results were reported for an experiment in which diploid and triploid *C. virginica* were dosed with *P. marinus* cells in a flume (Meyers et al. 1991). Tolerance to the effects of *P. marinus* must therefore be more specific than and unrelated to greater glycogen content associated with the lack of reproductive effort.

Although mortality comparisons between diploid and triploid groups were not made in this study, the similarity in susceptibility *P. marinus* suggests that mortalities would also be similar. This was confirmed by Meyers et al. (1991) who reported that cumulative mortality of *C. virginica* 150 days after being dosed with *P. marinus* was 100% and 98% for diploid and triploid groups, respectively.

The effect of *P. marinus* on oyster energy metabolism has not been examined to date, but it is recognized that *H. nelsoni* infection negatively affects oyster filtration rate, glycogen content, and condition (Newell 1985, Barber et al. 1988a, 1988b). Furthermore, oysters selected for tolerance of *H. nelsoni* exhibit greater filtration rates than susceptible oysters during periods of greatest parasite activity (Barber et al. 1991). Considering the inverse relationship between glycogen content and *H. nelsoni* infection intensity and the greater glycogen content of triploid oysters, an examination of the relationship between ploidy and infection of *C. virginica* by *H. nelsoni* would be a useful comparison to the findings presented here.

### Gametogenesis

As indicated by the significantly lower GAI values, triploid *C. virginica* exhibited considerably reduced gonadal development compared to diploids. While diploid oysters underwent a normal gametogenic cycle their second full summer (1990) and subsequently spawned mature gametes, more triploid oysters failed to differentiate, and those that were differentiated produced only a few isolated oocytes or ova (females) and spermatocytes (males, only one having spermatozoa). Based on histological observation, these immature gametes were resorbed, rather than spawned. Thus the little gametogenic material that was produced by triploids did not develop into significant numbers of gametes and was not spawned. These observations are consistent with those previously reported for *C. virginica* by Lee (1988).

Gametogenesis in triploid bivalves is either uninitiated because of the inability of homologous chromosomes to synapse in meiosis or arrested because of multivalent formation and aberrant segregation (Allen et al. 1986). In the cases of *C. virginica*, *A. irradians*, *Mya arenaria*, and *C. nobilis*, gametogenesis is initiated, but rarely completed (Tabarini 1984, Allen et al. 1986, Lee 1988, Komaru and Wada 1989). We found only one confirmed triploid

individual with spermatazoa, and few, if any, with advanced oocytes. Thus triploid *C. virginica* may rarely produce mature gametes. A population of triploid *C. virginica*, however, would be effectively sterile if mature gametes are produced only by males or if gametes, once produced by either sex, are not spawned.

In contrast to *C. virginica*, triploid *C. gigas* produced both spermatazoa and ova, although in smaller quantities than diploid siblings (Allen and Downing 1986, 1990). Although the mechanisms involved are not fully understood, the extent of gametogenesis in triploid *C. gigas* appears to be greater than in *C. virginica*. As noted by Allen and Downing (1990), spawning of both mature and immature gametes occurs in triploid *C. gigas*, and normal embryos are produced. The ability of triploid *C. gigas* to produce and spawn mature gametes, unlike the other species of marine bivalves examined to date, may be related to the tremendous fecundity displayed by this species (Allen and Downing 1986).

#### Commercial Use

Even though triploid *C. virginica* are no more disease resistant than diploids (Meyers et al. 1991, this study), they may have commercial value on the east coast of the U.S. in a hatchery-based aquaculture industry, selling to a "half-shell" market. As demonstrated in this study, placing oysters into the field in late summer (August) avoided heavy *P. marinus* infection the first year. The triploids, by virtue of their greater rate of growth, reached an

average shell height equal to commercial size (63.5 mm) by July of the second year, before *P. marinus* began causing mortality and reducing growth. Diploid oysters, on the other hand, did not average commercial size until November of the second year, by which time *P. marinus* had become well established and mortalities were numerous. Almost twice as many triploids were of commercial size in July 1990 at age 15–16 months than diploids, and the meat quality of the triploids was higher than that of diploids at this time of year, owing to differences in gonadal development.

As demonstrated in this study, the timing of placement of oysters in the field is important both with respect to disease and growth. By planting late the first year (August), infection by *P. marinus* can largely be avoided and growth of triploids to commercial size can be attained by July of the second year, prior to establishment of the parasite and subsequent mortality. These relationships may be site specific, however, and further comparisons are clearly warranted.

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## QUANTITATIVE GENETICS OF GROWTH IN THE DWARF SURFCLAM *MULINIA LATERALIS* (SAY, 1822)

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**ABSTRACT** Three spawnings of the dwarf surfclam *Mulinia lateralis* (Say) were conducted using a factorial mating design. Genetic and environmental factors affecting larval and juvenile growth were examined. Maternal effects on larval growth were evident in all spawns. Container effects appeared to be as important as genetic effects on larval growth. Both additive genetic effects (sire contributions) and non-additive genetic effects (sire  $\times$  dam interaction) on juvenile growth were detected in one spawn.

**KEY WORDS:** *Mulinia*, dwarf surfclam, genetics, growth

### INTRODUCTION

Because of its small size, short generation time, and relative ease of culture, the dwarf surfclam or coot clam *Mulinia lateralis* (Say, 1822) has been dubbed the "molluscan fruit fly" (Calabrese 1969a). Larvae can be reared to metamorphosis in 6-8 days at 25°C on an algal diet, and generation times of 39-135 days have been observed in the laboratory (Calabrese 1969a). In addition to studies on its ecology (Shumway and Newell 1984, Walker and Tenore 1984), developmental biology (Kidder 1972a, b, McLean 1976), and physiology (Calabrese 1969b, 1970, Kennedy et al. 1974, Shumway 1983), a number of recent works have focused on the relationship between genetic variation at enzyme-coding loci and fitness correlates such as growth rate and survival (Koehn et al. 1988, Gaffney et al. 1990, Scott and Koehn 1990). However, its promise as a model system for the quantitative genetics of bivalves remains largely unexplored. We report here the results of a preliminary study on the quantitative genetics of larval and juvenile growth in this species.

### MATERIALS AND METHODS

Three spawns were conducted. In each case, ripe adults were placed into separate finger bowls containing filtered seawater. After spawning was induced by thermal stimulation, gametes were collected and rinsed on a 100  $\mu$  mesh sieve, and resuspended in filtered seawater at 19°C. Eggs and sperm were combined in a factorial mating design, using more males than females in order to focus on genetic effects not confounded by effects of the maternal phenotype (e.g., egg ripeness or egg quality). Larvae were stocked at initial densities of 15-20 per ml and fed daily with 20,000 cells/ml of *Isochrysis galbana* and *Chaetoceros calcitrans*. Water was changed every second day and maintained at 20-25°C.

For Spawn A, adult broodstock were obtained in January 1989 from a hatchery line maintained by the Virginia Institute of Marine Science (Wachapreague, Virginia). After conditioning at the College of Marine Studies, two females and six males were crossed in April 1989 in a 2  $\times$  6 factorial mating design. One day after fertilization, each of the 12 resulting families was split into four batches. From each family two batches (replicates) were reared in the laboratory under relatively constant temperature and salinity; the other two replicates were reared in a greenhouse, where tem-

peratures fluctuated considerably. The greenhouse cultures were fed half the algal ration of the laboratory cultures, but this ration appears to have been supplemented by uncontrolled algal blooms. No attempt was made to control larval density. After metamorphosis, the spat from each pair of replicates were pooled and placed into a downwelling recirculating seawater system in the laboratory, at a density of 500 animals per 20 cm diameter basket. For each family-larval environment combination, the larval shell areas of 20 randomly selected animals were measured at one and three days after fertilization using a computerized image analysis system. Twenty juveniles per culture were measured to the nearest 0.1 mm at days 35 and 51 using digital calipers.

Spawn B was conducted with ripe wild-caught animals collected at Massey's Landing, Delaware in August 1989. Gametes from four females and five males were combined in a factorial design. For each family, 20 randomly chosen larvae were measured at one, five and ten days after fertilization. Larval density was not controlled. After metamorphosis, clams from each family were placed into two 20 cm baskets at a density of 200 per basket. One basket for each family was maintained in a downwelling recirculating seawater system at constant salinity (approx. 30 ppt), while the other was maintained in a similar downwelling recirculating seawater system with fluctuating salinity. Initially, the salinity was changed at 48-hour intervals, from 30 ppt to 22 ppt to 15 ppt; subsequent salinity changes were selected at random (i.e., at each water change, salinity was changed from the current level to either of the alternative salinities). This randomly fluctuating salinity was designed to impart a level of physiological stress that might reveal genotype-environment interactions. Shell lengths of 50 juveniles per culture were measured at 51, 65 and 76 days after fertilization. One of the twenty families was lost before any juvenile measurements were made. In order to make factorial ANOVA possible, we estimated the mean size for the missing family (Sokal and Rohlf 1981, p. 365) and used this estimate to represent a single individual sampled from the missing family for each sampling period.

For Spawn C, ripe wild-caught animals were collected at Massey's Landing, Delaware in April 1990. Gametes from three females and twelve males were combined in a partial factorial design to yield 24 families. One day after fertilization, each culture was thinned as necessary to a density of 15 larvae/ml. For each family, the shell areas of 20 randomly chosen larvae were measured at one and six days after fertilization. No further data were gathered, as none of these cultures survived to metamorphosis.

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Using a Model II (random effects) analysis of variance, we examined the components of variance in larval and juvenile size attributable to genotype and environment, with the Multivariate General Linear Hypothesis of SYSTAT (Wilkinson 1990). Genotypic effects were analyzed simply as differences among families, or when possible, as effects attributable to Sire (S), Dam (D) and  $S \times D$  interaction. For three-way models, first-order interaction terms were tested over the remainder mean square, while main effects were tested using the Satterthwaite approximation (Winer 1971). Because true replication was lacking (except for Day 3 of Spawn A), the highest-order interaction (i.e.,  $S \times D$  in a two-way model,  $S \times D \times E$  in a three-way model) was inevitably confounded with container effects. Therefore non-additive genetic effects ( $S \times D$  interaction) could be tested only in the three-way model, while genotype-environment interaction could not be evaluated. For the one replicated data set, a nested-factorial design was used, with replicates nested within families. Variance components for unbalanced nested designs were calculated according to Sokal and Rohlf (1981, p. 297). Larval survival data were arcsine transformed and densities square-root transformed before analysis (Sokal and Rohlf 1981).

## RESULTS

### Spawn A

At day 1, no significant Sire or Dam effects on larval size were evident; the interaction term, representing the confounded effects of  $S \times D$  interaction and among-container variation, was highly significant ( $P < 0.001$ ). No effect of S or D on larval density was apparent, although a Dam effect was suggested ( $P < 0.08$ ).

Because families were split into replicates after day 1, the next set of measurements (day 3) allowed container effects (variation between replicates within families) to be separated from genetic effects (variation among families). The loss of two families in the greenhouse prevented the estimation of a full nested-factorial ( $S, D, E$  and replicate within  $S \times D \times E$ ) model. However, a simplified model testing family, environment and replicate within family showed highly significant between-replicate variation ( $P < 0.005$ ), a modest family effect ( $P < 0.05$ ), and no effect of environment or family  $\times$  environment. In the lab cultures, among-family variation could be partitioned further into S, D and  $S \times D$  terms; none of these was significant.

Deleting families sired by males 2 and 3 allowed testing a full nested-factorial model, although with a reduced data set. Between-replicate variation was again highly significant ( $P < 0.00005$ ), and genotype-environment interaction was suggested ( $S \times D \times E, P < 0.10$ ). Of the main effects, only Dam was significant ( $P < 0.01$ ).

By day 35, the loss of five laboratory families precluded testing the full factorial ( $S \times D \times$  larval environment) model. Within each larval environment, no significant S or D effects were found; the  $S \times D$  term, representing non-additive genetic variance and/or container effects, was significant (lab:  $P < 0.06$ ; greenhouse:  $P < 0.001$ ). The simplified model (family  $\times$  environment) showed no significant effects of either family or environment, but a significant ( $P < 0.001$ ) interaction term (again, representing non-additive genetic variance and/or container effects) for both day 35 and day 51.

At day 1, culture density was not associated with mean larval size ( $r = 0.22, N = 12, NS$ ). By day three, mean larval shell area was positively correlated with culture density in the laboratory ( $r = 0.74, N = 22, P < 0.001$ ) and in the greenhouse ( $r = 0.61, N = 18, P = 0.007$ ; Figure 1). In addition, mean larval size was positively correlated with survival from day 1 to day 3 in the

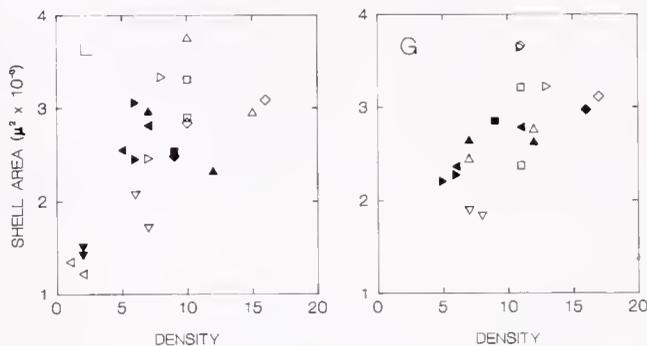


Figure 1. Spawn A. Mean larval size (shell area) in relation to density at day 3. L = laboratory; G = greenhouse. Open symbols = families of female 1; filled symbols = families of female 2. Symbol shapes indicate families of different males.

laboratory ( $r = 0.70, N = 22, P < 0.001$ ) but not in the greenhouse. No significant effects of S or D on survival from day 1 to day 3 were detected.

### Spawn B

At day one, a slight ( $P = 0.032$ ) Sire effect on larval shell area was detected, but was not evident at day 5 or day 10. No Dam effect was evident until day 10 ( $P = 0.010$ ). Culture density was not associated with larval size until day 10, when a negative correlation appeared ( $r = -0.77, N = 19, P < 0.001$ ; Figure 2). Density was clearly affected by Dam ( $P = 0.004$ ) but not Sire.

Three-way analysis of variance of juvenile size showed no Dam or Environment effect, but a slight ( $P < 0.10$ ) Sire effect at day 51, which later became more pronounced ( $P < 0.025$  and  $P < 0.01$  at days 65 and 76, respectively). The only significant interaction detected was  $S \times D$ , which was highly significant at day 51 ( $P < 0.0005$ ), day 65 ( $P < 0.0005$ ) and day 76 ( $P < 0.0025$ ). Environment had little effect on relative performance among families in juvenile growth; family means were highly correlated

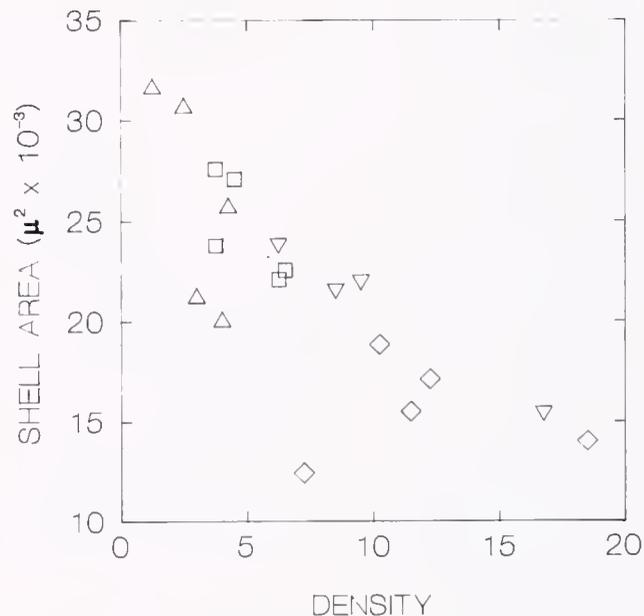


Figure 2. Spawn B. Mean larval size (shell area) in relation to density at day 10. Symbol shapes indicate families of different females.

across environments at day 51 ( $r = 0.97$ ,  $N = 19$ ,  $P < 0.001$ ), day 65 ( $r = 0.96$ ,  $N = 19$ ,  $P < 0.001$ ) and day 76 ( $r = 0.93$ ,  $N = 19$ ,  $P < 0.001$ ). Over time the  $S \times D \times E$  term became significant (day 51:  $P = 0.22$ ; day 65:  $P = 0.10$ ; day 76:  $P < 0.01$ ), but it is unclear whether this represented the gradual appearance of true genotype-environment interaction or merely container effects.

#### Spawn C

In this spawn, only larval size data were collected. Analysis of family means by two-way ANOVA without replication showed no Sire effect on either day one or day six, but a significant Dam effect at both times ( $P = 0.001$  and  $P < 0.00005$ , respectively). Since the role of density effects was presumably minimized by establishing uniformly low culture densities on day 1, the presence of a significant Dam effect and absence of a detectable Sire effect suggests a maternal effect on egg quality.

### DISCUSSION

In view of the relatively small scale of our experimental design, potential density effects and differential mortalities, we have not attempted to calculate conventional quantitative genetic measures such as heritability. In particular, the presence of significant between-replicate variation (container effects) observed in the only replicated data set suggests caution in interpreting the highest-order interaction term (i.e.,  $S \times D$  in a two-way model,  $S \times D \times E$  in a three-way model) in unreplicated cultures.

In all spawns, larval growth rates showed significant Dam but not Sire influences, emphasizing the role of maternal effects in early larval development. Both additive and non-additive genetic variance affecting juvenile growth were indicated by the significant Sire and Sire  $\times$  Dam effects observed in Spawn B. Factorial

mating schemes have revealed non-additive genetic variance in larval growth rate for *Crassostrea virginica* (Newkirk et al. 1977) and *Mytilus edulis* (Innes and Haley 1977, Newkirk et al. 1981) and in larval survival for *C. virginica* (Mallet and Haley 1984) and *C. gigas* (Lannan 1980).

A thorough analysis of genotype-environment interaction was generally not possible given the absence of true replication, i.e., two or more culture vessels for each family in each environment. However, the one replicated data set (Spawn A, Day 3, laboratory) can be used to gain some insight into the relative magnitudes of between-replicate (container) and genetic (family) effects. Container effects accounted for 22% and 18% of the total variance in size at day 3 in the lab and greenhouse cultures, respectively, while genotype accounted for 29% and 12%. The observed magnitude of container effects argues strongly for replicated designs in quantitative genetic analyses of larval growth.

Larvae were cultured at low densities in order to reduce the effects of differences in density on growth caused by differential survival. Nevertheless, significant relationships between mean size and culture density were detected, both positive (Spawn A, Day 3, laboratory) and negative (Spawn B, day 10). The former may occur when growth rate and survival covary positively, perhaps as a consequence of variation in egg quality, whereas the latter would be expected when egg quality is homogeneous and densities are high enough to hinder growth. While density may be held constant by regular culling, the variation in survival rates among families, particularly during the larval period, is an inescapable problem that may lead to the misinterpretation of genetic parameters or bias in their estimation. These problems may be minimized by including density as a covariate in quantitative genetic designs and by careful interpretation of density-related effects (e.g., Rawson and Hilbish 1990).

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## EFFECT OF NEUTRAL RED STAIN ON SETTLEMENT ABILITY OF OYSTER PEDIVELIGERS, *CRASSOSTREA VIRGINICA*<sup>1</sup>

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**ABSTRACT** The effect of neutral red stain on the settlement of oyster *Crassostrea virginica* (Gmelin) pediveligers was examined. Larvae were offered two types of substrate: oyster shell and acetate sheets. Settlement was measured as the proportion of pediveligers settled after 24 hours and analyzed with two-factor ANOVA. Staining did not significantly affect settlement, although settlement onto acetate was much lower than onto oyster shell.

**KEY WORDS:** vital stain, neutral red, larvae, settlement, competency, *Crassostrea virginica*

### INTRODUCTION

Studies of the early life history of bivalve mollusks often require examination and counting of larvae and newly-settled spat. Under some circumstances, this examination can be greatly facilitated by the use of a vital stain with the nearly transparent larvae or spat. It has been shown that a low concentration of neutral red stain colored oyster (*Crassostrea virginica*) larvae a bright red, with no apparent mortality or side effects (Loosanoff and Davis 1947, Manzi and Donnelly 1971).

In some cases, however, the swimming behavior of *C. virginica* pediveliger larvae in the laboratory was perceptibly altered for several hours after being stained with neutral red (unpublished data). Swimming rate decreased, compared to that of larvae without the stain, and there was a tendency for the stained larvae to clump. Because oyster settlement has a large behavioral component (Cranfield 1973), there is a concern that the use of neutral red to stain pediveliger larvae might affect their ability to settle. This article examines the effect of neutral red stain on the settlement of *C. virginica* eyed pediveliger larvae onto two types of substrates.

### MATERIALS AND METHODS

*Crassostrea virginica* pediveliger larvae were cultured in filtered York River water at 15 ppt salinity.<sup>2</sup> Visual examination of the larvae confirmed that more than 95% had both a foot and a large, distinct eyespot, which are considered morphological characteristics of larval competency to settle (Coon et al. 1985).

Manzi and Donnelly (1971) recommend culture concentrations of neutral red stain of 2.5-5.0 ppm for 24 hours in both distilled water and filtered seawater. In seawater however, neutral red stain forms a precipitate within several days, while solutions in distilled water can be kept at room temperature for months while no perceptible precipitation. Distilled water solutions were used in this experiment.

The larvae were held in 15 ml culture dishes (watch glasses), filled with 10 ml of 0.2 micron-filtered York River water at 15 ppt salinity. Salinities used by Loosanoff and Davis (1947) or Manzi and Donnelly (1971) were not given. Two settlement substrates were used: small, nearly flat *Crassostrea virginica* valves, and

circles of Mylar frosted acetate, both about 2.5 cm in diameter. The shells were scrubbed clean, the ligament was removed, and the concave surface was placed downward in the culture dish. The acetate circles were creased so that the frosted surface was concave, and the concave surface placed downwards in the culture dish. Mylar acetate has been used previously as a commercial settlement substrate (Dupuy et al. 1977).

Three days prior to the experiments, the shells and acetate circles were placed in a flowing seawater trough with adult *C. virginica*. Dissolved substances from both oyster shells (Vieth and Hidu 1971) and the common marine bacterium *Alteromonas* (Fitt et al. 1990) enhance settlement of oyster larvae. Exposure to seawater provided substrate accumulation of chemical substances which induce larval settlement. 3-4 Days of exposure permits optimal bacterial growth for settlement inducement (Fitt et al. 1990).

The experimental design involved a two-factor analysis of variance (Zar 1984). Factors were substrate treatment (shell versus acetate) and stain treatment (stain versus no stain), and each treatment combination had five replicates. Approximately 100 pediveliger larvae were added to each culture dish with a dropper pipette. Then three drops of the 0.1 ppt neutral red stain solution were added to the stain-treatment dishes, yielding a culture stain concentration of approximately 3 ppm. All of the culture dishes were covered with glass petri dishes, and covered with a dark cloth for 24 hours. The temperature was constant at 20°C.

At the end of 24 hours, the number of settled spat and free-swimming larvae were counted in each settlement chamber. There were no more than one or two dead larvae per culture dish; most of these had been accidentally crushed by handling. One culture dish was lost; the missing value was estimated using the Shearer technique (Zar 1984). Settlement was expressed as a proportion in each chamber. Prior to statistical analysis, each value was transformed by the arcsine square root method, to bring the data distribution closer to a normal distribution and satisfy the assumptions of analysis of variance (Zar 1984).

### RESULTS

Substrate type strongly affected settlement (Tables 1, 2). Mean proportional settlement on oyster shell was 64.2%, compared to only 1.4% on acetate. Staining did not significantly affect settlement (Tables 1, 2); the difference in mean proportional settlement between treatments was only 2.3%. The interaction effect was not significant (Table 2).

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<sup>2</sup>Virginia Institute of Marine Science oyster hatchery.

TABLE 1.

Settlement of oyster larvae, expressed as proportions of total larvae.

Oyster Shell		Mylar Acetate	
Stain	No Stain	Stain	No Stain
0.66	0.63	0.03	0.00
0.73	0.66	0.00	0.06
0.71	0.72	0.00	0.01*
0.36	0.82	0.00	0.03
0.82	0.31	0.01	0.00

\* Denotes estimated value; see text.

## DISCUSSION

The hypothesis that staining with neutral red affects settlement of *C. virginica* larvae was not substantiated, with an extremely small (2.3%) difference between staining treatments. The difference between proportional settlement on the substrate treatments (oyster shell versus acetate) shows the degree to which settlement of oyster larvae can be affected.

Although settlement was not affected by neutral red stain, there

TABLE 2.

Two factor analysis of variance.

Source	DF	SS	MS	F	p
Stain	1	0.0005	0.0005	0.022	0.887
Substrate	1	3.6607	3.6607	157.8	<0.0001
Interaction	1	0.0079	0.0079	0.341	0.586
Error	16	0.3713	0.0232		
Total	19	4.0403			

may be effects on metamorphosis (a physiological event separate from settlement) or spat survival or growth. Metamorphic success and post metamorphic survival is affected strongly by physiological stress (Baker and Mann, in press).

Manzi and Donnelly (1971) examined the effects of neutral red and other stains on larvae of *C. virginica* and the venerid clam *Mercenaria mercenaria*, and reported no difference in survival and growth rates between the two species. *Mercenaria* or other bivalve pediveligers were not used in this study, but based upon Manzi and Donnelly's work, it is reasonable to infer that neutral red will have no significant effect on proportional settlement on other bivalve mollusk taxa.

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## ANALYSIS OF LARVAL OYSTER GRAZING BY FLOW CYTOMETRY<sup>1</sup>

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**ABSTRACT** The ingestion of 8 algal species by oyster larvae (*Crassostrea gigas* Thunberg, 1793) was measured by flow cytometry (FCM). In a preliminary experiment, cell size (estimated by light scatter) and chlorophyll fluorescence of 30 algal species were evaluated to select those species which could be mixed together and still be easily discriminated by FCM. Grazing experiments were carried out over 48 h with 6 and 15-day old larvae fed on 3 algal mixtures, each containing 3 different algal species. The concentration of each species was estimated at 0, 6, 12, 24 and 48 h by FCM. Grazing pressure on a given algal species was dependent upon the age of the larvae, the time of the day and the composition of the mixture. Grazing rates of older larvae were about twice those of younger ones after 48 h (mean value of 102 and 57 cells/larva/hour respectively). Almost no grazing activity was observed during the time interval 12–24 h for the 6-day larvae. Significant differences between mixtures were observed after 48 h and the selective filtration of one *Chaetoceros* strain is of importance as this alga also proved to be of better nutritional value for oyster larvae. Data on *Tetraselmis* were difficult to interpret because of tigmotactic reactions of the cells.

**KEY WORDS:** oyster larvae, *Crassostrea gigas*, grazing, algae, selective feeding, flow cytometry

### INTRODUCTION

The type and amount of food used during the rearing process are among the most important factors necessary to bring large numbers of oyster larvae to metamorphosis (Rhodes and Landers 1973, Gerdes 1983). Cole (1937) was the first to demonstrate that the use of marine unicellular algae could produce significant growth of *Ostrea edulis* L. larvae under laboratory conditions and cultures of unicellular algae are still used commonly in hatcheries and nurseries rearing young bivalve molluscs (Ukeles 1976, Chrétiennot-Dinet et al. 1986). Three criteria need to be fulfilled for an alga to be used in a bivalve nursery: adequate size, good food quality and ease of mass culturing. Different species have been tested for their food quality (see for example Walne 1970, Nascimento 1980, Wikfors et al. 1984, Enright et al. 1986, Whyte 1987), and *Isochrysis galbana* is often used as a reference standard for the growth response of bivalves (Gerdes 1983). It has also been demonstrated that mixtures of several algal species generally improve larval growth (Guillard 1975, Epifanio 1979, Gerdes 1983). On the other hand, Reid (1982) suggested that suspension feeders in culture may have specific requirements and that their feeding behavior must be assessed species by species. Ukeles (1976) pointed out that oyster larvae seem to have more specific dietary requirements than clam larvae and that juveniles are less demanding than veligers. Recent studies have examined the capacity of bivalves to select nutritive particles from inorganic material of

similar size (Kjørboe et al. 1980, Kjørboe and Møhlenberg 1981, Newell and Jordan 1983, Newell et al. 1989). One important criterion for studying such selection is the ability to recognize particles and cells of similar size but different quality. A major advantage of flow cytometric analysis (FCM) is the ability to distinguish simultaneously between cells and particles of nearly equal dimensions but of different optical properties. For these reasons, FCM has been introduced recently to study the grazing of filter-feeding organisms (Cucci et al. 1985, 1989, Shumway et al. 1985, 1990). Experiments on feeding selectivity have been performed mostly on adults (Shumway et al. 1985) and little information is available for oyster larvae (Korringa 1952, Crisp et al. 1985). Mackie's results (1969) suggest that species selectivity is effective within a given size range but according to Crisp et al. (1985), oyster veliger larvae (mean size over 200  $\mu\text{m}$ ) do not discriminate between algal species or algal sizes when fed on mixtures of *Nannochloris*, *Isochrysis* and *Tetraselmis*. Fritz et al. (1989), working with natural assemblages of phytoplankton, concluded that larvae select small cells (<10  $\mu\text{m}$ ) but that little selection occurs within this size range. In the present paper, we describe the use of FCM to detect possible particle selection by oyster larvae grazing up on mixtures of various algal species.

### MATERIAL AND METHODS

#### Flow Cytometry

In order to choose phytoplankton species suitable for grazing experiments, 30 cultures (Table 1) were first analysed in Roscoff with an EPICS 541 flow cytometer (Coulter, Hialeah, Florida).

<sup>1</sup>This work was part of the fourth GAP (Group for Aquatic Productivity) meeting in L'Houmeau (France): 19–22 April 1988.

TABLE 1.

List of the species screened by flow cytometry with their origin, their average size and the symbol used in Fig. 1. The last column indicates the mixture in which they have been used during the grazing experiment.

Species	Source	Size (µm)	Symbol (Fig. 1)	Mixture where used
<i>Synechococcus</i> sp. (ROSO4)	Roscoff (F)	1-2	1	
<i>Micromonas pusilla</i> (ROSO9)	Roscoff (F)	1-2	2	
Flagellate 1	Argenton (F)	2-3	3	
Flagellate 2	Argenton (F)	2-3	4	
<i>Microcystis</i> sp.	Arcachon (F)	2-3	5	
<i>Stichococcus bacillaris</i>	Arcachon (F)	3-6	6	
<i>Pseudoisochrysis paradoxa</i>	USA	4-6	7	
<i>Nannochloris atomus</i>	Arcachon (F)	2-3	8	
<i>Nannochloropsis salina</i>	Marseille (F)	2-3	9	
<i>Chaetoceros</i> "minus"	Tahiti (F)	3-4	10	A
<i>Chaetoceros pumilum</i>	Conway (U.K.)	3-8	11	B
Flagellate 3 (ROSO7)	Roscoff (F)	3-4	12	
Flagellate 4	Argenton (F)	3-4	13	
<i>Chroomonas</i> sp.	Arcachon (F)	10-13	14	
<i>Isochrysis</i> "tahiti"	Tahiti (F)	5-6	15	C
<i>Hemiselmis</i> sp.	L'Houmeau (F)	4-5	16	
<i>Thalassiosira pseudonana</i>	Arcachon (F)	3-5	17	
<i>Chlamydomonas</i> sp.	Arcachon (F)	6-10	18	
<i>Chaetoceros calcitrans</i>	Brest (F)	5-16	19	
<i>Pavlova lutheri</i>	Brest (F)	7-9	20	
<i>Isochrysis galbana</i>	Conway (U.K.)	5-6	21	A,B
<i>Chaetoceros gracilis</i>	Tahiti (F)	6-10	22	
<i>Dunaliella primolecta</i>	Roscoff (F)	7-9	23	
<i>Tetraselmis mcisa</i>	Arcachon (F)	8-10	24	A
<i>Tetraselmis tetrathele</i>	Arcachon (F)	10-16	25	B
<i>Pleurochrysis carterae</i>	USA	9-12	26	
<i>Cryptomonas maculata</i>	Roscoff (F)	9-14	27	
<i>Tetraselmis suecica</i>	Conway (U.K.)	9-11	28	C
<i>Gymnodinium</i> cf. <i>nagasakiense</i>	Roscoff (F)	25-30	29	
<i>Pyramimonas disomata</i>	Argenton (F)	4-6	30	C

Excitation light was provided by an argon laser tuned at 488 nm (100 mW). From 1000 to 10000 cells were analysed for the following parameters: (i) forward angle light scatter (FALS), (ii) right angle light scatter (RALS), (iii) orange fluorescence (OFL: between 530 and 590 nm) which is proportional to phycoerythrin, (iv) red fluorescence (RFL: above 690 nm) which is proportional to chlorophyll. For each species the average value of each parameter was computed and standardized to that of fluorescent beads added to the sample. FALS and RALS were standardized to 1 µm beads (Polysciences) while OFL and RFL were standardized to 10 µm beads (Coulter: 2% of fullbright).

Among the analyzed species (Table 1), some were easily discriminated by FCM because of specific features such as the orange fluorescence of phycoerythrin for cyanobacteria and cryptophytes or the high ration of RALS to FALS for cocco lithophorids (Olson et al. 1989); others had sufficiently different scatter and chlorophyll signatures to be distinguished (Fig. 1). When two species were too similar, however, there was an overlap between the two populations and it was not possible to assign those cells with intermediate properties to one or the other species. For a given difference in average cell properties between two species, the population overlap is lower when individual cells differ little from the average cell, i.e. if there is little spread in the population. A practical rule would be that the population means + 2 population standard deviations do not overlap:

$$x_1 + 2\sigma_1 < x_2 - 2\sigma_2 \quad (1)$$

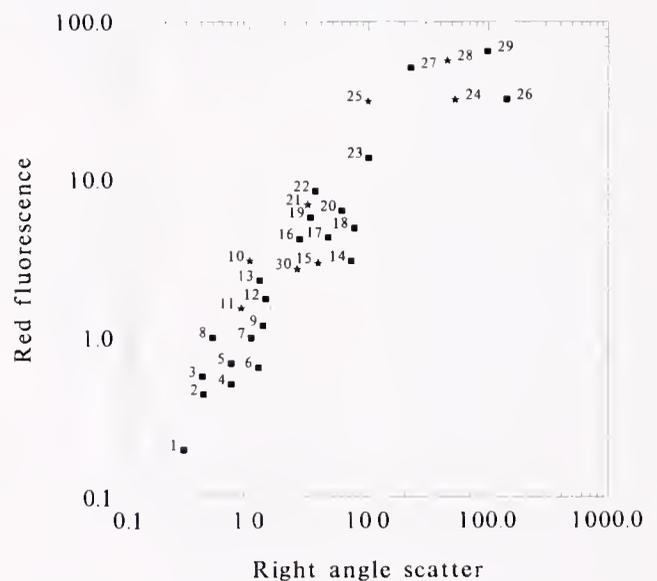


Figure 1. Flow cytometric measurements of the average right angle scatter (related to 1 µm beads) and red fluorescence above 690 nm (related to 10 µm 2% beads) for 30 phytoplankton species. Stars correspond to selected species used in mixture (see Table 1 for the list of species).

where  $x_1$  and  $x_2$  are population averages of a given cellular property for two species (1 and 2) and  $\sigma_1$  and  $\sigma_2$ , their population standard deviations. Assuming that the two species have the same population coefficient of variation (C), this would give:

$$x_1 + 2 Cx_1 < x_2 - 2 Cx_2 \quad (2)$$

or

$$x_1/x_2 < (1 - 2C)/(1 + 2C) \quad (3)$$

Although population spread varied widely depending on species and culture status (exponentially growing cultures were in general more tightly distributed than stationary phase ones), the population coefficient of variation was, in general, of the order of 30%. Therefore two species could usually be discriminated if their ratio for at least one of their average properties was larger than 4 (equation 3).

In view of this analysis and of practical experimental constraints (resistance of the cells to handling, adaptability to mass culture), three mixtures of three species were used for the grazing experiments (Table 1, Figs. 1 and 2); a last change had to be made in mixture C as a small cryptophyte culture collapsed during handling just at the beginning of the experiments and had to be replaced by *Pyramimonas disomata*. Unfortunately the latter species could not be discriminated from *Isochrysis* "tahiti" and the two species had to be counted together. However the results of mixture C can be compared to those of mixtures A and B for total grazing activity.

During the grazing experiments, a 0.1 ml volume was analysed for each sample with an EPICS Profile (Coulter). Excitation was provided by an argon laser (488 nm, 20 mW). Measured param-

eters were identical to those for the selection of species except for orange fluorescence which was detected between 515 and 640 nm. Each parameter was recorded on a 3 decade logarithmic scale mapped onto 1024 channels. Data acquisition was done in list mode. The Profile software supplied the volume analyzed and the concentration of each species in the sample.

#### Algal Cultures and Oyster Larvae

The eight algal strains used in the experiments (Table 1) were chosen not only according to their flow cytometric properties, but also to test their potential food value, i.e., their ability to be grazed. They belong to different taxonomic groups and some are well known as food sources for bivalve larvae (Walne 1970, Nascimento 1980, Whyte 1987). Among them *Isochrysis galbana* is considered to be among the best foods for oyster larvae (Guillard 1958, Webb and Chu 1982), but many are local isolates and need testing. Each culture was grown at 18°C in Conway's medium (Walne 1966) and illuminated by fluorescent tubes giving a mean surface irradiance of  $50 \mu\text{Eins} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  with a light-dark cycle of 14:10. Cultures were in exponential phase at the beginning of the experiments.

Larvae of the Japanese oyster, *Crassostrea gigas* Gmelin (6 and 15-day old) were provided by IFREMER-LA TREMBLADE (France) and kept unfed in the dark at 22°C for 24 hours prior to the grazing experiments. Their mean size was  $94.4 \pm 0.9 \mu\text{m}$  and  $125.8 \pm 3.2 \mu\text{m}$  respectively.

Three algal mixtures (A, B, C), each containing 3 different algal species (Table 1 & Fig. 3A, B, C), were fed to the larvae. Each mixture was tested in duplicate on both 6 and 15 day-old larvae, as well as in the absence of larvae (control). For each test, 5000 larvae were placed in 2 litre spherical flasks filled with 1 litre of 0.2  $\mu\text{g}$  filtered seawater (salinity = 28‰). Algal mixtures were added to these flasks at the beginning of the experiment. The final concentration of each algal species was  $10^4 \text{ cells} \cdot \text{ml}^{-1}$ . Flasks were kept in the dark at 22°C. Samples of 10 ml, prefiltered on a 100  $\mu\text{m}$  mesh inox grid to remove the larvae, were taken at times  $t = 0, 6, 12, 24$  and 48 hours.

#### Data Analysis

The grazing pressure is expressed either as the number of cells of each species remaining in the medium at the different sampling times (on the basis of  $10000 \text{ cells} \cdot \text{ml}^{-1}$  at  $t = 0$ ) (Fig. 3); as the number of cells cleared per larva per hour for each species (Fig. 4); or as the number of cells cleared per larva in the different mixtures (Fig. 5). The use of a control rather than  $t = 0$  densities accounted for any change in cell density from  $t = 0$  which occurred in the absence of larvae. The grazing activity between times  $t$  and  $t'$  (G: number of cells cleared per larva per hour) was computed as:

$$G = (X'_n(t) - X'_n(t'))/L \cdot (t - t')$$

where,

$$X'_n(t) = 10000 \cdot X_n(t)/Y_n(t)$$

with  $X_n(t)$  the number of cells per ml of species  $n$  at time  $t$  in the grazed sample counted by FCM,  $Y_n(t)$  the number of cells in the control and  $L$  the number of larvae per ml.

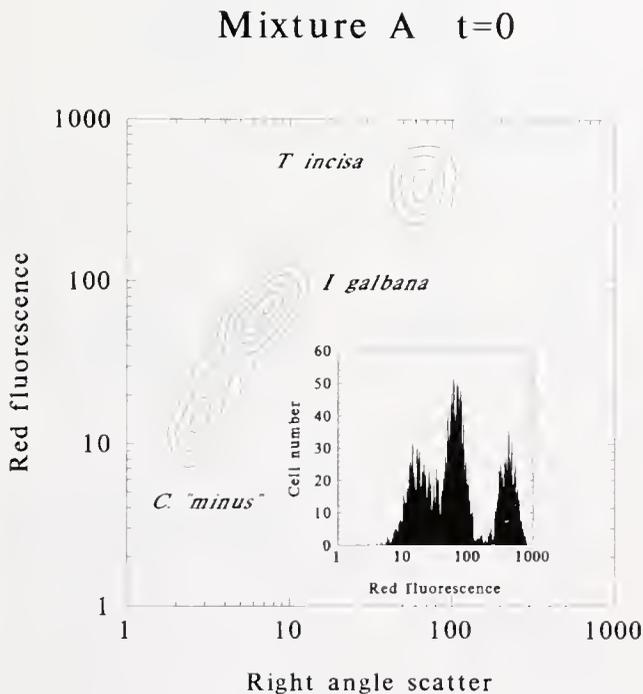


Figure 2. Cytogram of right angle scatter vs red fluorescence (above 690 nm) for mixture A at time  $t = 0$  of the grazing experiment. Axes are scaled in arbitrary units. Contours correspond to 2, 5, 10, 15 and 20 cells. The inset represents the one-dimensional histogram of red fluorescence for this sample. It reveals that *Chaetoceros* "minus" and *Isochrysis galbana* are less well separated than *Tetraselmis incisa*.

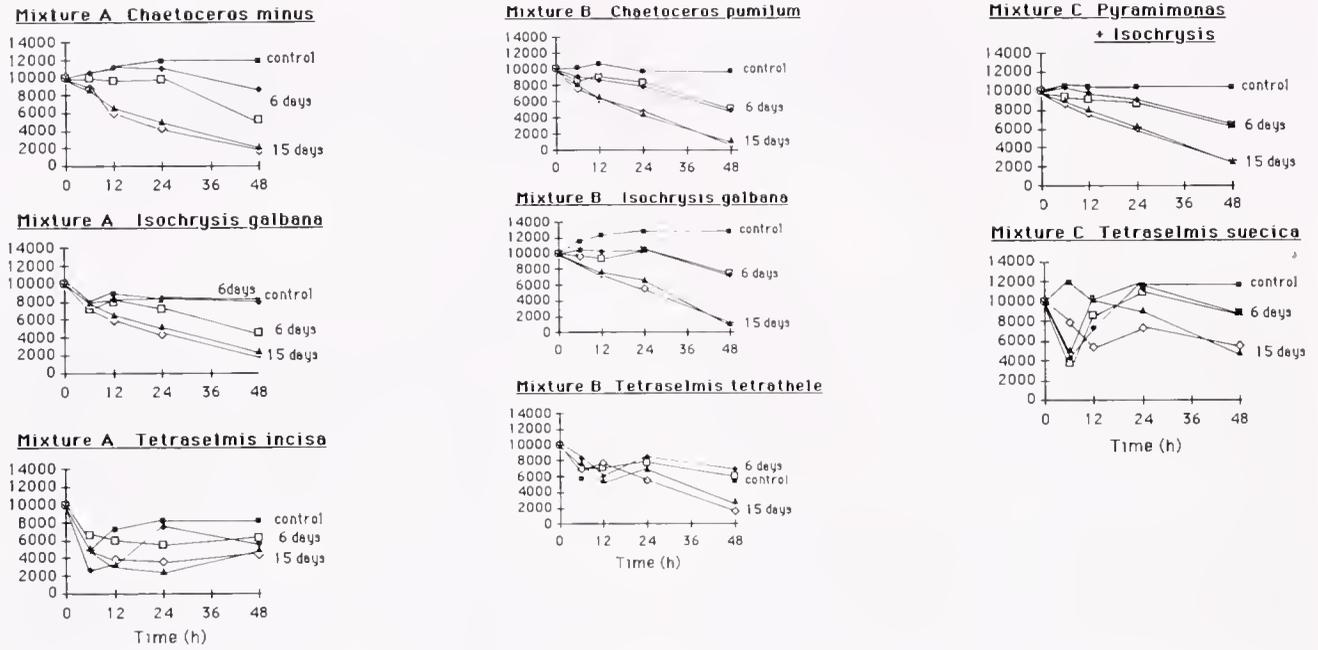


Figure 3. Evolution of cell number of each species vs time, for both types of larvae (6 and 15 day old) and for the control (different symbols denote replicate experiments).

RESULTS AND DISCUSSION

Selection of food species is critical for rearing experiments and for successful hatchery culture (Guillard 1958). According to Webb and Chu (1982), and our own experience, all species used in the present experiment were potential food sources for oyster larvae. *Tetraselmis* spp. were introduced in the mixtures because of their relatively large size as compared to the mouth diameter of the youngest larvae (Ukeles and Sweeney 1969, Robert and His 1987). Cell concentration as well as larval densities are factors affecting feeding activity (Schulte 1975, Ukeles 1976) and care was taken to keep larval densities low enough (5 larva per ml) to ensure high individual feeding activity, but high enough to expect significant variations with an initial cell concentration of  $3 \cdot 10^4$  cells  $\cdot$  ml<sup>-1</sup> at the beginning of the experiments (Nascimento, 1980, gives 20–30 cells  $\cdot$   $\mu$ l<sup>-1</sup> as an adequate concentration for *Crassostrea gigas* larvae of 70–80  $\mu$ m in length). The larvae generally grazed actively as large numbers of cells were cleared from the medium after 48 h for both types of larvae (Fig. 3). The response of both replicates is quite similar for the 6 and 15 day-old larvae, except for *Tetraselmis* (see explanation below) and for mixture A with 6 day-old larvae which were less active in one sample. However, grazing rates varied among the different species, depending on larval age, time, and mixture composition (Fig. 4).

From a quantitative point of view, larval age (related to size) is most important. After 48 h, (Fig. 4G and 5C), older larvae (15-day) had cleared roughly twice as many cells as younger ones: on average 102 and 57 cells in a mixture per larva, per hour, respectively. This result is in agreement with the fact that the amount of food ingested increases with larval size (Rhodes and Landers 1973, Lucas and Rangel 1981, Gerdes 1983). The difference between both ages of larvae was most evident during the time interval 6–12 h for mixture A, as the amount of cells cleared by the 15-day larvae was 6 times higher than for the 6-day larvae (Fig. 4B) and greater still in the three mixtures after 12 h (Fig. 4E and 5B).

The time sequence of the number of cells cleared (Fig. 3, 4 and 5) shows clearly that the grazing pressure was not constant during the experiment. Both groups of larvae grazed actively during the first 12 h, except for the 6-day old larvae with mixture A (Fig. 4A

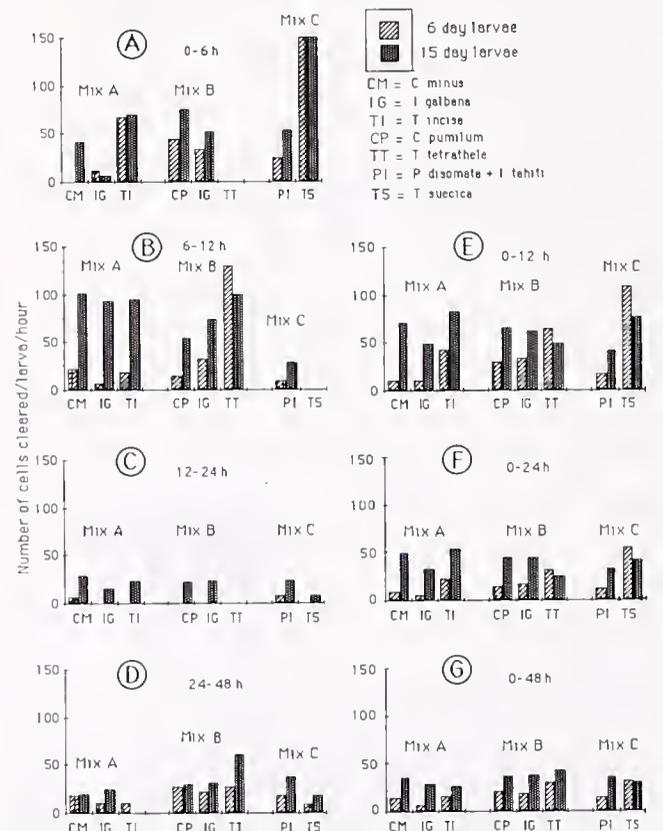
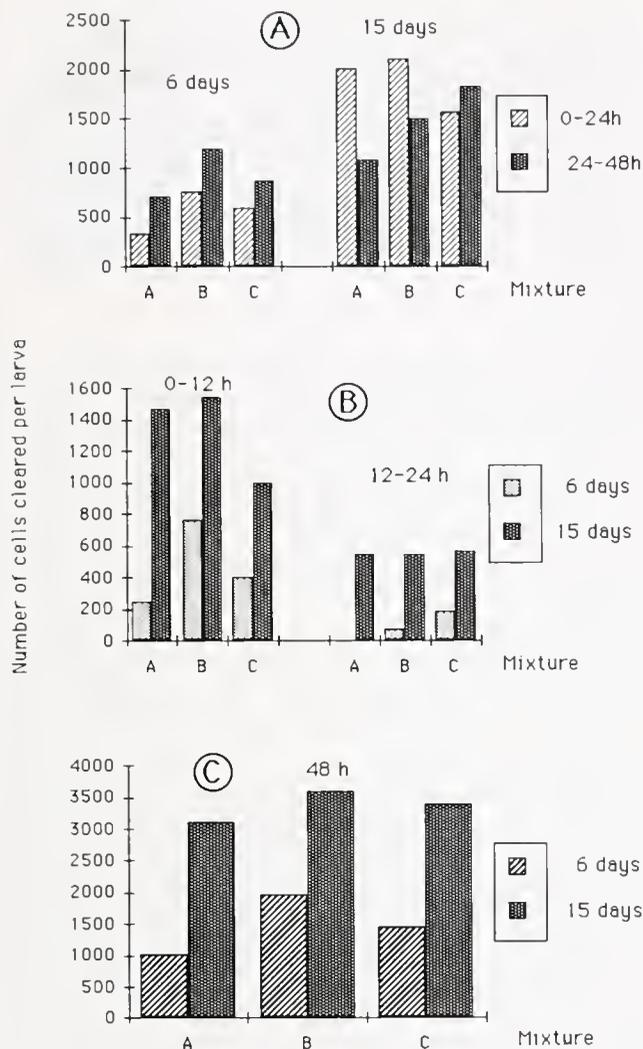


Figure 4. Number of cells cleared per larva and per hour for the different species at various time intervals: mean value of the duplicates.



**Figure 5.** Number of cells cleared per larva in the different mixtures (*Tetraselmis* values discarded): mean value of the duplicates. A: comparison between the first (0–24 h) and second day (24–48 h) for the 6 and 15 day-old larvae; B: comparison between the 6 and 15 day-old larvae during the time intervals 0–12 and 12–24 h; C: comparison after 48 h between the 6 and 15 day-old larvae.

and B, Fig. 5B). Between 12 and 24 h, the grazing pressure was much lower; reduced to a third of the 0–12 h values for the 15-day larvae and almost none for the 6-day ones (Fig. 4C and Fig. 5B). It seems that the larvae filled their stomachs within the first 12 hours, and then reduced their grazing activity considerably. This type of behavior has already been observed in prosobranch veligers (Fretter and Montgomery 1968) and rhythmic activity has been demonstrated in adults of *Ostrea edulis* (Morton 1971) or *Crassostrea virginica* (Palmer 1980).

Under natural conditions, information on larval feeding behavior is rather scarce (His et al. 1985, Robert and His 1988) but experimental studies usually indicate a continuous feeding strategy (Korringa 1955, Ukeles 1976, Fritz et al. 1984). A comparison between the 1st and 2nd day (Fig. 4F, D and Fig. 5A) shows that the 6-day larvae grazed more on the 2nd day, (1.5 times more on average), but that older ones were less active, except for mixture C. A two-way analysis of variance (ANOVA) after 48 h (*Tetraselmis* values excluded) shows significant differences between the

6 and 15-day old larvae ( $p = 0.999$ ) but also between species ( $p = 0.988$ ).

A comparison between the different mixtures after 48 h (Fig. 5C) shows that grazing activity is highest with mixture B and lowest with mixture A for both types of larvae. During the time interval 0–6 h, *Chaetoceros "minus"* (mixture A) was not grazed at all by the 6-day larvae, while *Chaetoceros pumilum* (mixture B), of a similar size, was apparently consumed readily (Fig. 4A). *Isochrysis galbana* was also less grazed in mixture A than in mixture B (Fig. 4A, B and D). It is of interest to note that mixtures A and B contained two different species of *Chaetoceros* whose effect on the growth and survival of oyster larvae has been tested with special emphasis on their lipid contents (Robert et al. 1989). The best growth, obtained with *C. pumilum*, seems to be explained by the optimal balance of the different lipid classes and fatty acids as the lipid content of larvae is clearly related to the diet. *Chaetoceros "minus"* is then considered to be of rather poor food value and the lack of feeding by the 6 day larvae on this alga might be related to its chemical composition. It is also clear that larvae ingest what is available and without other alternative, ingested mixture A on the 2nd day. Feeding behavior is a complex phenomenon with possible adaptations relating to environmental conditions, but younger larvae are more sensitive to the mixture composition and different species belonging to the same genus may not prove to be equivalent. The demonstration of a relationship between particle selection and biochemical nutritional value for *Chaetoceros pumilum* is of great interest for larval bivalve feeding studies as growth is tightly related to food ingestion and FCM is a promising tool from this point of view. The case of *Tetraselmis* has to be considered separately since after 6 h, cells were observed to attach to the vessel wall at the air-water interface. As a result of this tigmotactic behaviour, the cell density had drastically decreased in the 6 h samples, giving a high apparent grazing activity on the species of this genus. It was then decided to agitate the flasks before sampling. The phenomenon probably biased some grazing estimates as the results were somewhat erratic (Fig. 3) and *Tetraselmis* data were discarded for the ANOVA and in values given in Fig. 5. However, after 48 h, the mean number of each type of cell (all species included) cleared per larva and per hour did not differ significantly whether *Tetraselmis* data were included or not: 19 vs 15 for the 6 day larvae and 34 vs 35 for the 15 day larvae (Fig. 4). Because of these problems, it was not possible to discuss the influence of the cell size of *Tetraselmis* on the grazing activity of the youngest larvae.

## CONCLUSIONS

FCM is useful for the study of selective grazing by bivalve larvae fed on mixed algal populations, provided suitable species are available in culture. The first 12 hours are particularly sensitive for selectivity, and mixture composition is more critical for smaller larvae. Care must be taken under experimental conditions using algal cultures to select phytoplankton cells which can be distinguished by FCM, but it is also important to avoid cells which tend to clump or stick, sink or aggregate. In this respect, *Tetraselmis* is not recommended for FCM analysis. The selected algae must also be easy to grow, resistant to handling (fragile species cannot be used) and nontoxic. For young larvae, algal cells must be of a suitable size, usually less than 10  $\mu\text{m}$ , therefore the range of species becomes rather restricted. About 50 species belonging to 35 genera have been tested under experimental conditions on bivalve larvae (Chrétiennot-Dinet et al. 1986). However, marine

phytoplankton comprises more than 50 known genera with representatives within the size range 1–5  $\mu\text{m}$  and almost 100 within 5–10  $\mu\text{m}$ . Only 3 algal genera in the size range considered are reported to have toxic effects in aquaculture (Shumway 1990). It would be very interesting in the future to try to apply FCM to *in situ* analysis, as the natural diet of bivalve larvae is still poorly known and our knowledge of the natural food content of young bivalve larvae very limited (His et al. 1985, Chrétiennot-Dinet and Guillocheau 1987, Robert and His 1988).

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# EFFECTS OF MESH SIZE, STOCKING DENSITY AND DEPTH ON THE GROWTH AND SURVIVAL OF PEARL NET CULTURED BAY SCALLOPS, *ARGOPECTEN IRRADIANS CONCENTRICUS*, IN SHRIMP PONDS IN SOUTH CAROLINA, U.S.A.

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**ABSTRACT** The effects of mesh size, stocking density, and depth on growth and survival of the southern bay scallop, *Argopecten irradians concentricus*, cultured in pearl nets in a penaeid shrimp pond at the Waddell Mariculture Center in Bluffton, S.C. (USA) were examined. Three mesh size treatments (N = 5 pearl nets per treatment), i.e., 3 mm, 6 mm, and 9 mm mesh were tested (50 scallops per net;  $\bar{x}$  = 12.5 ± 0.2 mm shell length). Four density treatments (6 mm mesh; N = 9 pearl nets per treatment) were tested i.e., 25, 50, 75, and 100 scallops per net ( $\bar{x}$  = 18.1 ± 0.14 mm shell length). All nets (~0.09 m<sup>2</sup>) were placed in a 0.1 hectare shrimp pond.

Poor survival (18%) was observed in the smallest (3 mm) mesh nets. Survival was significantly higher in 6 mm (65%) and 9 mm (77%) mesh nets. Results were complicated by water column depth effects. No scallops survived in the four bottom 3 mm mesh nets or the one bottom 6 mm mesh nets. While survival was significantly higher in the upper nets in two mesh treatments (i.e., 3 mm and 6 mm), no significant difference in survival occurred between upper (N = 3) and lower (N = 2) 9 mm mesh nets. Growth increased with mesh size. A significant difference was detected in scallop size between upper ( $\bar{x}$  = 51 mm) and lower ( $\bar{x}$  = 52.3 mm) nets for the 9 mm mesh net trial.

After 20 weeks, no significant difference in survival occurred between experimental densities or between upper versus lower nets for the four experimental densities. After 20 weeks, mean size was greatest at the 25 stocking density ( $\bar{x}$  = 52.3 mm), >50 stocking density ( $\bar{x}$  = 49.9 mm) >75 stocking density ( $\bar{x}$  = 48.9 mm) = 100 stocking density ( $\bar{x}$  = 47.9 mm). No significant difference in scallop mean size was detected between upper and lower nets for any of the experimental densities tested at the 20 week mark.

**KEY WORDS:** *Argopecten irradians*, scallops, ponds, density, pearl nets, mesh size, growth, survival, biculture

## INTRODUCTION

Attempts to grow the southern bay scallop, *Argopecten irradians concentricus*, and the northern bay scallop, *Argopecten irradians irradians*, under field conditions in the coastal waters of Georgia have met with limited success (Heffernan et al. 1988, Walker et al. 1991). The subspecies, *A. i. irradians*, was unable to survive high summer water temperatures and both subspecies became heavily fouled with oyster spat when suspended in estuaries which hindered scallop growth and survival.

Attempts were made to control oyster fouling in *A. i. concentricus* by growing scallops within tanks (4270 l) at the Shellfish Research Laboratory, Skidaway Island, Georgia and in a penaeid shrimp pond at the Waddell Mariculture Center in Bluffton, S.C. (Walker et al. 1991). Virtually no oyster fouling occurred on scallops within the tank system, but limited fouling occurred on scallops in the shrimp pond. At present, mariculturists cannot culture scallops economically in a tank system due to pumping costs, algae production costs, etc. However, the use of penaeid shrimp ponds offers an excellent opportunity for producing a supplementary crop in conjunction with penaeid shrimp.

This study examines the effects of pearl net mesh size, stocking density, and water column depth position on growth and survival of *A. i. concentricus* in a penaeid shrimp pond.

## METHODOLOGY

All bay scallops used in this study were spawned in the Shellfish Research Laboratory at Skidaway Island, Georgia. Parental stocks were initially obtained from St. Joseph Bay, Florida in 1987. The scallops used for the density experiment were spawned from F<sub>1</sub> progeny on October 22, 1988, and had obtained a mean size of 12.4 ± 0.2 (SE) mm by June 29, 1989. Scallops used for the pearl net mesh size experiment were from F<sub>1</sub> generation spawnings from October 22, 1988 to November 16, 1988. These scallops were combined and screened through a 20 mm sieve to remove the largest scallops and then through a 5 mm sieve to remove the smallest. The mean shell length (ear to ear axis) of these remaining animals was 18.1 ± 0.14 mm.

To determine the effect of density and mesh size on growth and survival of scallops, 25, 50, 75, and 100 scallops of a mean size of 12.5 ± 0.2 mm were placed in 9 pearl nets of 6 mm mesh each. Floor surface area of a pearl net is 0.09 m<sup>2</sup>. Scallops (N = 50) of a mean size of 18.1 ± 0.14 mm were placed in 5 nets each of 3 mm, 6 mm, and 9 mm mesh size. Nets were tied two to a line and suspended, one above the other, from floats in a shrimp pond (0.1 hectare) at Waddell Mariculture Center, Bluffton, South Carolina, on June 30, 1989.

The ponds are generally stocked with penaeid shrimp from the

end of April to the first of May and are harvested from the last part of October to the first week of November. Water within the ponds, which have a maximum depth of 1.2 m, is aerated and circulated by a paddle wheel.

Growth and survival were monitored at weeks 10 and 20. Each net was opened and a sample of 30 or all (the 25 density nets) specimens were measured for shell length with Vernier calipers. A total live count was made, scallops were returned to appropriate pearl nets and resuspended in the pond.

Data sets were analyzed by Analysis of Variance Tests (ANOVA) and Tukeys Multiple Range Test using SPSS software. All percentage data were arcsine transformed prior to statistical analysis.

## RESULTS

After 10 weeks, no significant difference (as determined by ANOVA) in scallop survival was detected among mesh treatments for nets in which some scallops survived (Table 1). However, after 20 weeks, survival was significantly less in the 3 mm mesh nets, and there was no survival in lower nets of the two smaller mesh sizes (3 mm and 6 mm). After 10 and 20 weeks of growth, significant differences in scallop size were detected in the different mesh sizes. At 20 weeks, Tukey multiple range tests showed that scallops grown in the 9 mm mesh nets ( $\bar{x} = 51.5$  mm) were significantly larger than those in the 6 mm mesh nets ( $\bar{x} = 49.8$  mm) which were significantly larger than those in the 3 mm mesh nets ( $\bar{x} = 42.5$  mm).

After 10 weeks, significant differences (as determined by ANOVA) in survival of scallops planted at various densities were detected (Table 2). At 10 weeks, Tukey multiple range test results reveal that differences occurred among the 25 and 50 scallops per net stocks, with no difference among the other treatments. At 20 weeks, no significant difference in survival among densities occurred. Significant differences (as determined by ANOVA) in mean size among densities were detected at 10 and 20 weeks. At

10 weeks, Tukey multiple range tests showed that mean size was greatest at the lowest density, with no significant differences observed between the 50 and 75 density nets or between the 75 and 100 density nets. At 20 weeks, scallop size was greatest at 25 ( $\bar{x} = 52.3$  mm) > 50 ( $\bar{x} = 49.9$  mm) > 75 ( $\bar{x} = 48.9$  mm) = 100 ( $\bar{x} = 47.9$  mm) scallops per net.

Results of the ANOVA tests on size and survival data for scallops cultured in the various mesh sizes according to water column position were run only for scallops cultured in the 9 mm mesh nets, since there were no survivors in either the 3 mm or 6 mm pearl nets located on the lower row at week 20. No significant difference in survival or size occurred for scallops grown in the upper versus lower nets at 10 weeks. Scallops were significantly larger in the lower nets at 20 weeks.

No significant differences in survival or size between upper versus lower nets were detected for any density trial at 20 weeks, however significant differences occurred in the size data for the 50 and 100 density trials at 10 weeks. In both cases, scallops cultured in top nets of the 50 ( $\bar{x} = 43.2$  mm) and 100 ( $\bar{x} = 42.2$  mm) density trials, were significantly larger than those in bottom nets ( $\bar{x} = 41.6$  mm and 40.2 mm, respectively).

## DISCUSSION

Results of this study show a direct correlation between scallop growth and both mesh size of pearl nets and stocking density. Greater mesh size and lower stocking density produced greater scallop growth. Our density-dependent size data agree with observations from Duggan (1973) and Rhodes and Widman (1980) (see Table 3). Duggan (1973), using densities similar to ours, but pens (9 mm mesh) rather than nets, reported a direct correlation between increases in scallop growth and decreases in stocking densities. Rhodes and Widman (1980) showed a similar density-dependent growth response, when comparing scallop growth in lantern nets (using densities of 500, 1000, and 1500 per m<sup>2</sup>).

TABLE 1.

The mean size, number of bay scallops, *Argopecten irradians concentricus*, and depth position per net used to determine the effects of pearl net mesh size upon growth and survival of scallops cultured in a penaeid shrimp pond.

June 30, 1989		September 8, 1989		November 17, 1989		
$\bar{x} \pm SE$ in mm	(No.)	Position	$\bar{x} \pm SE$ in mm	(No.)	$\bar{x} \pm SE$ in mm	(No.)
3 mm mesh						
18.2 ± 0.4	50	Lower	37.7 ± 0.53	50	—	0
18.4 ± 0.4	50	Upper	37.0 ± 1.16	50	42.5 ± 0.54	46
17.5 ± 0.3	50	Lower	35.5 ± 0.63	49	—	0
17.7 ± 0.3	50	Lower	35.8 ± 0.44	50	—	0
18.8 ± 0.3	50	Lower	37.2 ± 0.51	48	—	0
6 mm mesh						
19.0 ± 0.4	50	Lower	44.2 ± 0.55	50	—	0
19.0 ± 0.4	50	Upper	46.2 ± 0.52	48	51.1 ± 0.57	42
17.0 ± 0.2	50	Upper	43.4 ± 0.56	50	49.4 ± 0.58	38
18.1 ± 0.4	50	Upper	44.7 ± 0.49	48	49.5 ± 0.51	40
17.5 ± 0.3	50	Upper	43.8 ± 0.53	48	49.4 ± 0.65	43
9 mm mesh						
18.4 ± 0.3	50	Upper	45.4 ± 0.57	48	50.1 ± 0.78	30
17.8 ± 0.2	50	Upper	45.7 ± 0.54	50	51.1 ± 0.50	39
18.2 ± 0.3	50	Lower	45.5 ± 0.54	49	51.9 ± 0.21	46
18.1 ± 0.3	50	Upper	46.5 ± 0.49	48	51.8 ± 0.54	41
18.1 ± 0.3	50	Lower	47.1 ± 0.52	42	52.7 ± 0.48	36

TABLE 2.

The mean size, number of scallops, and water column position per net used to determine the effects of stocking density upon growth and survival of bay scallops cultured in 6 mm mesh pearl nets suspended in a penaeid shrimp pond.

June 30, 1989			September 8, 1989		November 17, 1989	
$\bar{x} \pm SE$ in mm	(No.)	Position	$\bar{x} \pm SE$ in mm	(No.)	$\bar{x} \pm SE$ in mm	(No.)
12.5 ± 0.2	25	Upper	44.7 ± 0.83	13	53.3 ± 1.14	12
12.5 ± 0.2	25	Lower	42.5 ± 0.69	13	50.4 ± 1.38	9
12.5 ± 0.2	25	Upper	45.6 ± 0.59	12	52.4 ± 0.78	11
12.5 ± 0.2	25	Lower	46.0 ± 0.91	15	53.4 ± 0.92	15
12.5 ± 0.2	25	Upper	43.5 ± 0.58	14	51.4 ± 0.90	12
12.5 ± 0.2	25	Lower	44.3 ± 1.40	15	0	0
12.5 ± 0.2	25	Upper	45.3 ± 0.85	13	52.9 ± 1.28	11
12.5 ± 0.2	25	Lower	44.8 ± 0.81	16	52.6 ± 1.09	13
12.5 ± 0.2	25	Upper	44.3 ± 1.12	16	51.7 ± 0.97	14
12.5 ± 0.2	50	Upper	43.4 ± 0.67	40	49.0 ± 0.91	33
12.5 ± 0.2	50	Upper	42.6 ± 0.79	39	49.9 ± 0.43	39
12.5 ± 0.2	50	Lower	43.6 ± 0.51	?	50.1 ± 1.10	15
12.5 ± 0.2	50	Lower	42.9 ± 0.65	32	50.3 ± 0.81	35
12.5 ± 0.2	50	Upper	42.9 ± 0.98	36	50.7 ± 0.56	34
12.5 ± 0.2	50	Lower	40.7 ± 0.73	38	0	0
12.5 ± 0.2	50	Lower	42.0 ± 0.71	41	51.3 ± 0.70	26
12.5 ± 0.2	50	Upper	*	*	50.3 ± 0.73	33
12.5 ± 0.2	50	Lower	*	*	48.1 ± 0.77	31
12.5 ± 0.2	75	Lower	43.4 ± 0.98	40	47.8 ± 0.47	53
12.5 ± 0.2	75	Upper	42.9 ± 0.65	53	48.4 ± 0.73	48
12.5 ± 0.2	75	Lower	41.0 ± 0.85	63	47.9 ± 0.76	35
12.5 ± 0.2	75	Upper	41.3 ± 0.76	64	49.4 ± 0.88	40
12.5 ± 0.2	75	Upper	42.0 ± 0.83	48	49.4 ± 0.92	47
12.5 ± 0.2	75	Upper	42.0 ± 1.07	28	49.8 ± 0.89	54
12.5 ± 0.2	75	Lower	42.1 ± 0.65	63	0	0
12.5 ± 0.2	75	Upper	42.2 ± 0.87	56	49.0 ± 0.30	40
12.5 ± 0.2	75	Lower	39.5 ± 0.89	45	49.8 ± 1.56	24
12.5 ± 0.2	100	Lower	38.2 ± 0.80	77	48.9 ± 0.60	42
12.5 ± 0.2	100	Upper	42.0 ± 0.77	78	49.7 ± 0.75	45
12.5 ± 0.2	100	Upper	40.5 ± 0.78	58	48.6 ± 0.94	39
12.5 ± 0.2	100	Lower	40.9 ± 0.63	70	49.0 ± 0.85	31
12.5 ± 0.2	100	Upper	41.4 ± 0.94	49	46.7 ± 0.63	69
12.5 ± 0.2	100	Lower	41.0 ± 0.49	63	46.3 ± 0.94	50
12.5 ± 0.2	100	Upper	43.6 ± 0.66	55	47.8 ± 0.60	59
12.5 ± 0.2	100	Lower	42.4 ± 0.83	54	45.5 ± 0.82	7
12.5 ± 0.2	100	Lower	39.8 ± 0.69	63	46.7 ± 0.61	65

\* Nets missed during sampling.

There have been no studies performed to our knowledge which examined the effects of mesh size used (in the protection system) on the growth and survival of scallops. *Mercenaria mercenaria* seed cultured in four different mesh cages (3 mm, 6 mm, 12 mm, and 19 mm) grew best in the smallest mesh cages, whereas *Spisula solidissima* grew best in the largest mesh cages (Walker and Heffernan 1990). It is clear from this study that scallops grew better in the larger mesh size nets.

Growth rates of scallops in this study are higher than those reported in previous studies in Georgia and South Carolina (Heffernan et al. 1988, Walker et al. 1991) (Table 3) but are comparable to growth rates reported for cultured scallops in more northern areas (Castagna and Duggan 1971, Duggan 1973, Rhodes and Widman 1980). Scallops (N = 50) grown in the largest mesh size nets had the greatest growth rate (8.7 mm per month), while those grown at lowest densities (25 scallops per net) also had the greatest growth rate (7.6 mm per month). The difference in the observed growth rates for the various Georgia and South Carolina studies

are most likely explained by stocking densities and subspecies strain utilized. Experiments with the northern bay scallop (*Argopecten irradians irradians*) grown in southern areas (Heffernan et al. 1988) yielded the slowest observed growth rates (range of 3.2 to 4.9 mm per month). Northern bay scallops were undoubtedly stressed due to their inability to tolerate warmer summer water temperatures, and eventually died (Heffernan et al. 1988). The low values reported for the southern bay scallop (*Argopecten irradians concentricus*) cultured in Georgia and South Carolina experiments ranged from 6.1 to 7.1 mm per month (Walker et al. 1991). These scallops were initially cultured at 100 scallops per net and after 10 weeks, densities were halved (Walker et al. 1991). Thus, stocking densities may account for the lower observed growth rates, since those values (range of 6.1 to 7.1 mm per month) are comparable to values observed at higher densities in this study (6.6 to 7.1 mm per month) (Table 3). However, differences in species and abundance of phytoplankton and culture conditions between years could affect growth rates.

TABLE 3.

Growth rate of bay scallops, *Argopecten irradians*, under culture conditions from various areas of coastal United States.

Location/Culture System	Initial Size in mm	Final Size in mm	Time Months	mm per Month	Source
Long Island Sound, CT					
Latern Net					
500/m <sup>2</sup>	24	46.2	3.0	7.4	Rhodes & Widman 1980
1000/m <sup>2</sup>	24	41.7	3.0	5.9	
1500/m <sup>2</sup>	24	40.1	3.0	5.4	
Wachapreague, VA					
Floating Pen	16.1	57.4	5.0	8.3	Castagna & Duggan 1971
Wachapreague, VA					
Floating Pen					
Surface	14.4	44.7	4.0	7.6	Duggan 1973
1m	14.4	44.6	4.0	7.6	
2m	14.4	47.0	4.0	8.2	
Bottom	14.4	42.7	4.0	7.1	
25/0.09 m <sup>2</sup>	14.4	46.2	4.0	8.0	
50/0.09 m <sup>2</sup>	14.4	43.5	4.0	7.3	
75/0.09 m <sup>2</sup>	14.4	39.0	4.0	6.2	
100/0.09 m <sup>2</sup>	14.4	37.0	4.0	5.7	
Wassaw Sound, GA					
Pearl Nets					
<i>Priest Landing</i>					
Surface 100/net	6.5	36.0	8.0	3.7	Heffernan al. 1988
Subtidal 100/net	6.5	33.2	8.0	3.7	
200/net	6.5	32.2	8.0	3.2	
<i>House Creek</i>					
Surface 100/net	6.5	37.4	8.0	3.9	
200/net	6.5	37.9	8.0	4.0	
Surface 70/net	9.8	49.0	8.0	4.9	
Wassaw Sound, GA					
Tank <sup>1</sup>	11.4	44.5	4.7	7.1	Walker et al. 1991
Tank <sup>2</sup>	11.4	41.4	4.7	6.5	
Pearl Nets <sup>3</sup>	11.4	40.7	4.7	6.3	
Pearl Nets <sup>4</sup>	11.4	42.8	4.7	6.8	
Bluffton, SC					
Pearl Nets <sup>5</sup>	11.4	42.8	4.7	6.5	
Cage <sup>6</sup>	11.3	39.9	4.7	6.1	
Pearl Nets <sup>7</sup>	11.3	42.9	4.7	6.8	
Bluffton, SC					
Pearl Nets					
3 mm	12.5	42.5	4.5	6.7	This paper
6 mm	12.5	49.8	4.5	8.3	
9 mm	12.5	51.5	4.5	8.7	
25/net	18.1	52.3	4.5	7.6	
50/net	18.1	49.9	4.5	7.1	
75/net	18.1	48.9	4.5	6.8	
100/net	18.1	47.9	4.5	6.6	

<sup>1</sup> Pearl nets in buried tank.<sup>2</sup> Pearl nets in tank on concrete pad.<sup>3</sup> Pearl nets at House Creek.<sup>4</sup> Pearl nets at Priest Landing.<sup>5</sup> Pearl nets in a penaeid shrimp pond.<sup>6,7</sup> Pearl nets and cages suspended in penaeid shrimp pond.

The observed mortality patterns in this density study differed from results obtained by Duggan (1973), but were similar to those of Rhodes and Widman (1980). We observed no significant difference in survival among the four stocking densities. Although Duggan (1973) performed no statistical tests on his data, a clear trend showing decreases in mortality with decreasing initial stocking density was evident. Percent mortalities for the stocking den-

sities of 100, 75, 50, and 25 scallops per 0.09 m<sup>2</sup> were 35%, 16%, 6.2%, and 3.2%, respectively (Duggan 1973). Although Rhodes and Widman (1980) provided no data, they stated that of the three stocking densities tested (500, 1000, and 1,500 per m<sup>2</sup>), significant mortalities occurred only at the highest densities. Our largest stocking densities of 100 scallops per net was approximately equal to their 1000 scallops per m<sup>2</sup>.

Significantly fewer scallops survived in the 3 mm mesh nets compared to other mesh size nets. These results are complicated by the water column net position factor. While the position of upper versus lower nets had no effect on the 9 mm mesh nets, a profound effect was observed in the smaller mesh size nets. No scallops survived in the lower layer of the 3 mm or 6 mm mesh nets. Eliminating the lower nets from the data set results in 92% survival in the 3 mm nets compared to 82% and 73% for the 6 mm and 9 mm mesh nets, respectively. In other mesh cage experiments, no significant differences in mortality were detected for *Spisula solidissima* or *Mercenaria mercenaria* (except at the largest mesh size for *Mercenaria mercenaria*, where clams were initially smaller than the cage mesh size and were washed out of the enclosure by tidal currents) when planted in four different mesh size cages (Walker and Heffernan 1991).

The cause of mortalities in the lower nets for the 3 mm and 6 mm nets during this study remains unknown. This problem is further complicated by the fact that comparable mortalities did not occur within the lower layer of the density experiment which were also in 6 mm mesh nets. There was no clear/consistent relationship between position of net (laterally) and the observed vertical mortality pattern. Duggan (1973) observed a marked increase in mortality of scallops located one meter above the river bottom (29%) and surface (16.5%) as compared to 8% and 4% for scallops suspended one and two meters below the surface. If some environmental parameter associated with the pond bottom or river bottom is responsible for the observed mortalities, then significant mortalities should have been observed in all lower nets.

Scallop pearl net culture (see Aoyama 1989) is labor intensive. Initially seed scallops are placed in the smallest mesh nets (3 mm) and as the juveniles grow, moved into a series of larger mesh size

nets (6 mm then to 9 mm). Final growout occurs when scallops are placed in lantern nets. The changing of nets ensures ample water with food passes through the nets. The longer the time interval between changes, the greater the fouling of nets by epibenthic organisms. The more fouling, the less water movement through the nets. Yet each changing of the nets requires labor input. By placing scallops in 9 mm mesh nets, commercial size scallops can be cultured within shrimp ponds without changing nets.

Whether the observed decrease in fouling (pond nets vs. river nets) is due to fouling organisms not migrating into the ponds or that the shrimp effectively reduce fouling organisms by grazing is unknown. In the absence of heavy fouling, nets do not have to be changed. The results of these experiments show that scallops grow, but survive poorly when kept in the smallest mesh size bag. However, good growth and survival were exhibited in the two larger mesh size pearl nets.

One of the objectives of this study was to determine if scallops could be cultured in shrimp ponds as a supplementary crop to the penaeid shrimp crop. Results of this work show that it is biologically feasible to culture bay scallops with penaeid shrimp. Economic feasibility is still unknown.

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## AN ASSESSMENT OF STRATEGIES FOR GROWING MUSSELS IN SUSPENDED CULTURE

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**ABSTRACT** Strategies for growing mussels in suspended culture were evaluated at two commercial operations on the Atlantic coast of Nova Scotia, Canada. Specifically, the study examined how the yield of mussels per sleeve was influenced by site, line position, seed density, sleeving material, and the use of sticks to reduce losses of mussels. At harvest, the average number of mussels per sleeve was similar between sites, but one site had a higher shell weight per unit length which translated into a higher yield per sleeve (9.5 vs. 8.0 kg). Shell and tissue growth were similar for mussels suspended from surface and subsurface lines, but lower losses at depth meant a higher yield, up to 1.7 kg more per sleeve. The proportion of mussels lost from the sleeves ranged from 35 to 50%, with the medium seed density (400 mussels/m of sleeve) showing the least loss. Line position and seed density also influenced the intensity of secondary settlement; the ratio of juveniles to adults was lower on the subsurface lines, and lowest on the sleeves with the highest mussel density. The type of sleeving material and the presence or absence of sticks did not affect yield, but did affect the economic return per sleeve.

**KEY WORDS:** *Mytilus edulis*, suspended culture, growth, mortality

### INTRODUCTION

Mussels produced in Atlantic Canada are typically grown in sleeves suspended in the water column from a longline system. Although the basic principles of suspended culture are well-established, specific husbandry practices and materials may vary substantially among farms. This variation partly reflects the adaptation of methods to local conditions, but more importantly, it reflects the continual development of new and innovative strategies. Those producers who wish to remain competitive in the rapidly expanding industry must determine which of these strategies will maximize their economic return.

This study was designed to evaluate grow-out procedures for suspended culture from both a biological and an economic perspective. Yield per sleeve, or the weight of mussels obtained at harvest, is the net result of increases in biomass due to growth and losses in biomass due to mortality and fall-off. Previous studies have suggested that growth and mortality, and thus ultimately yield, are affected by site (Mallet et al. 1986, 1987, Mallet and Carver 1989), grow-out depth (Kautsky 1982), and seed density (Kautsky 1982, Boromthananat and Deslous-Paoli 1988). In addition to these factors, we investigated the effect of different types of sleeving material on yield and the value of using sticks to reduce mussel fall-off. At the end of the study, estimates of the revenue obtained per sleeve were divided by the cost of the sleeving material, the mussel seed, and the sticks to determine those strategies with the highest economic return.

### MATERIALS AND METHODS

#### Experimental Sites

The two experimental sites were located approximately 100 km south of Halifax, on the Atlantic coast of Nova Scotia. The first was Indian Point Marine Farms, located at Indian Point near the town of Mahone Bay, and the second was Corkum's Island Mussel Farm, located in Upper South Cove just outside the town of Lunenburg. The two sites were selected for their very different environmental characteristics (pers. obs.). The Mahone Bay site

was relatively exposed with depths of 20 m, little mussel recruitment, a stratified water column during the summer, and little ice coverage during the winter. In contrast, the Lunenburg site was protected, with depths no greater than 6 m, heavy mussel settlement, little stratification, and 3 to 4 months of ice coverage.

To ensure that the results of this study were directly relevant to the mussel industry, standard (i.e. 1988) culture techniques were used wherever possible. Initially, two parallel 100-m longlines were established at each site (Fig. 1). After attaching the experimental sleeves, one line in each pair was sunk using sandbags; 2 m below the surface at Lunenburg and 10 m below the surface at Mahone Bay. The variation in the depth of the subsurface lines between sites reflected differences in the standard procedures used by the two mussel growers. From a statistical perspective, the specific depths of the subsurface lines were not important; rather, the sleeves were considered either "exposed" (surface) or "sheltered" (subsurface) from wave action. Note that in order to avoid ice damage, the surface lines were sunk to roughly the same depths as the subsurface lines at the end of December.

#### Sleeving of Juvenile Mussels

In late May 1988, approximately 50 70-l tubs of mussel seed (30 mm) were obtained from collectors at Lunenburg and used as a seed source for both sites. At each site 16 different types of sleeves (24 replicates of each) were prepared:

- (1) Dupont sleeving material (20 × 20 mm mesh): 200, 400, and 600 mussels/m, with and without sticks;
- (2) Italian sleeving material (20 × 20 mm mesh): 200, 400, and 600 mussels/m, with and without sticks;
- (3) Fresh Klean sleeving material (10 × 10 mm mesh): 200 and 400 mussels/m, with and without sticks.

The three seed densities were also referred to as low (200 mussels/m), medium (400 mussels/m) and high (600 mussels/m). All sleeves were 3 m in length, while the total number of mussels per sleeve varied from 600 to 1800, with the exception of the Fresh Klean sleeves which were too narrow to accommodate 1800 mussels. The "sticks" were made of 1.6-cm diameter, 30-cm long

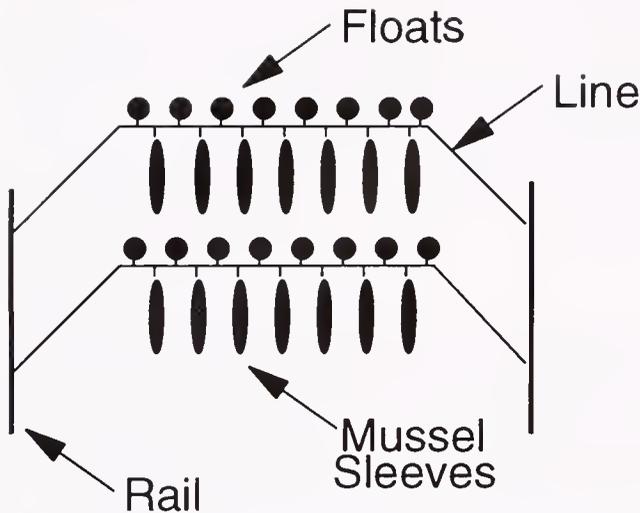


Figure 1. Diagram of the two parallel mussel lines set up at each site; one line in the pair was sunk below the surface at the beginning of the study.

polyethylene pipe; three of these were inserted at regular intervals along the sleeve in order to reduce the fall-off or loss of mussels.

The sleeves were left in shallow water for 2 to 3 h in order to promote byssal attachment, and were then suspended from the two lines at each site (12 replicates of each type, or 192 sleeves per line). To reduce the likelihood of a position effect, each line was divided into 12 blocks and 1 replicate of each type was randomly assigned to 1 of the 16 positions within each block. Records of the material costs incurred with the different treatments were later used in calculating the net economic return per sleeve.

#### Sampling Procedure

Initial sampling of the sleeves at Lunenburg and Mahone Bay was carried out on June 9 and 10, respectively. This involved taking 2 replicates of each sleeve type from randomly designated

positions along each line for a total of 4 replicates of each type per site. The total volume of mussels removed from each sleeve was recorded and a 1-*l* sample was frozen for later analysis.

On October 18, 2 replicates of each sleeve type were again harvested from each of the two lines at each site. The volume of mussels removed from each sleeve was recorded and a 2-*l* sample was taken from each replicate and frozen. The same sampling procedure was carried out at Lunenburg on December 9 and Mahone Bay on December 29. Growth of mussels at Lunenburg over this 20-day period would have amounted to only 0.1 mm (Mallet and Carver, unpubl. data). Poor weather conditions and logistical problems were responsible for the delay in the sampling schedule.

The final harvest was carried out on April 24 at Mahone Bay and April 28 at Lunenburg. The volume of each of the remaining socks was measured and 5-*l* samples were taken from 2 replicates of each type. The mussels in each sample were counted and the lengths of 50 animals were recorded. Five of these 50 animals were chosen at random, dissected and dried at 60°C to give estimates of dry tissue weight and shell weight. In addition, the volume of newly-settled juveniles on the Lunenburg sleeves was determined in October, December and April.

#### Data Analysis

Growth was estimated from the change in shell length, dry shell weight per unit length, and dry tissue weight per unit length over each interval. Wet tissue weight per mussel was calculated from dry tissue weight multiplied by a factor of 5 (Mallet and Carver 1989). The number of mussels per sleeve was estimated from the total volume in litres multiplied by the appropriate number of mussels/*l* which varied depending on their size (June: 266, October: 71, December: 58, April: 40).

Production was calculated by the method of Allen (1951). For each type of sleeve, the number of survivors was plotted against mean weight for each sampling interval. Daily production (g/sleeve/day) was calculated as the average number of animals present during an interval multiplied by the weight increment for that interval divided by the appropriate number of days. Final

TABLE 1.

Repeated measures analysis of variance of mean shell length, mean shell weight per unit length, mean dry tissue weight per unit length, and volume to test the effect of various treatments.

Source	DF	Shell Length	Shell Weight	Tissue Weight	Volume
		(mm)	(mg/mm)	(mg/mm)	(l)
		MS	MS ( $\times 10^{-6}$ )	MS ( $\times 10^{-6}$ )	MS
Site	1	40.98*	1269.5*	101.5*	0.92
Position	1	0.06	149.6*	91.9*	481.8*
Density	2	5.29	74.9	37.4*	1571.6*
Sleeve type	2	4.04	44.5	6.5	83.6
Sticks	1	0.01	52.9	1.0	8.0
Site $\times$ position	1	37.39*	355.3*	17.2*	46.2
Material $\times$ density	3	3.41	40.1	2.5	77.0
Material $\times$ sticks	2	1.07	6.5	1.6	10.3
Density $\times$ sticks	2	1.09	5.1	1.3	1.7
Initial values	1	1.69	36.5	4.3	
Error	47	1.96	32.2	63.6	1.82

MS refers to the mean square value and DF denotes degrees of freedom. \* indicates a significant effect at  $p < 0.05$ .

TABLE 2.

Least square means (X) and standard error (SE) of shell length (mm), shell weight per unit length (mg/mm), and tissue weight per unit length (mg/mm) of mussels from the surface and subsurface lines at Lunenburg and Mahone Bay in June 1988, October 1988, December 1988 and April 1989.

	Lunenburg				Mahone Bay			
	Surface		Subsurface		Surface		Subsurface	
	X	SE	X	SE	X	SE	X	SE
Shell Length (mm)								
Jun 88	33.5	0.5	33.5	0.5	30.2	0.4	30.2	0.4
Oct 88	49.0	0.5	49.9	0.3	47.7	0.3	46.0	0.3
Dec 88	51.8	0.3	52.0	0.4	50.9	0.4	51.1	0.4
Apr 89	56.6	0.3	58.1	0.2	56.7	0.2	55.5	0.3
Shell Weight (mg/mm)								
Jun 88	26.0	1.4	26.0	1.4	25.7	0.9	25.7	0.9
Oct 88	58.6	1.6	66.7	1.4	56.1	1.3	55.2	1.2
Dec 88	68.3	1.5	68.9	1.4	64.4	1.6	64.5	1.4
Apr 89	74.8	0.9	79.6	1.8	73.8	1.4	71.8	1.4
Tissue Weight (mg/mm)								
Jun 88	7.5	0.4	7.5	0.4	7.3	0.2	7.3	0.2
Oct 88	14.8	0.5	14.3	0.4	11.3	0.3	16.1	0.4
Dec 88	13.7	0.4	13.8	0.3	14.6	0.5	16.1	0.5
Apr 89	21.6	0.5	24.4	0.5	26.8	0.7	26.4	0.6

yield, or weight of mussels per sleeve at the time of harvest, was determined by multiplying the number of mussels per sleeve in April 1989 by their average whole wet weight (shell plus wet tissue).

To estimate the economic return per sleeve, the yield of market-size mussels at harvest (kg) was multiplied by the typical market price of \$1.10 per kg to give revenue per sleeve (all prices listed are in 1988 Canadian dollars). This was then divided by the cost per sleeve or, in other words, the sum of the initial seed cost, the cost of the sleeving material, and where applicable, the cost of sticks which included a labour component. Other labour costs were not included as these were effectively the same for all sleeve types.

The effect of the various treatments (i.e. site, line position, seed density, sleeving type, and sticks) on growth (shell length, tissue weight and shell weight), mortality/fall-off, volume, production, yield and economic return per sleeve was tested by means of multivariate repeated measures analyses of variance. The repeated measurements consisted of the seasonal values for each variable.

## RESULTS

### *Biological Assessment of the Grow-out Strategies*

#### (a) Shell and Tissue Growth

Site and site by position had a significant effect on growth in terms of shell length (Table 1), whereas position, density, sleeving material type, sticks, and their interactions were not important. Note that initial differences in shell length did not significantly affect the final values. At the time of harvest in April, the mean shell length was 57.1 mm at Lunenburg versus 55.9 mm at Mahone Bay (Table 2).

The only treatments which had a significant effect on growth in terms of shell weight per unit length were site, position, and site by position (Table 1). Differences in initial shell weight per unit length were not significant. In April, the average shell weight of a 55-mm mussel was 4.2 g at Lunenburg versus 4.0 g at Mahone Bay (Table 2). The mussels with the highest shell weight came from the subsurface line at Lunenburg; 44.4 g for a 55-mm animal.

TABLE 3.

Least square means (X) and standard error (SE) of volumes (l/sleeve) of mussels averaged over site, sleeving material and sticks. The three seed densities (200, 400, and 600) are in mussels/m of sleeve.

Density	Surface						Subsurface					
	200		400		600		200		400		600	
	X	SE	X	SE	X	SE	X	SE	X	SE	X	SE
Jun 88	3.0	0.1	5.2	0.2	7.2	0.5	3.0	0.1	5.2	0.2	7.2	0.5
Oct 88	6.6	0.5	11.5	0.5	13.7	1.7	7.6	0.3	13.8	0.6	17.6	2.5
Dec 88	6.4	0.8	12.4	1.0	13.6	1.3	7.7	0.5	16.9	0.8	18.0	1.4
Apr 89	8.2	0.9	17.0	1.0	18.2	1.6	10.2	1.0	21.1	1.2	25.2	2.3

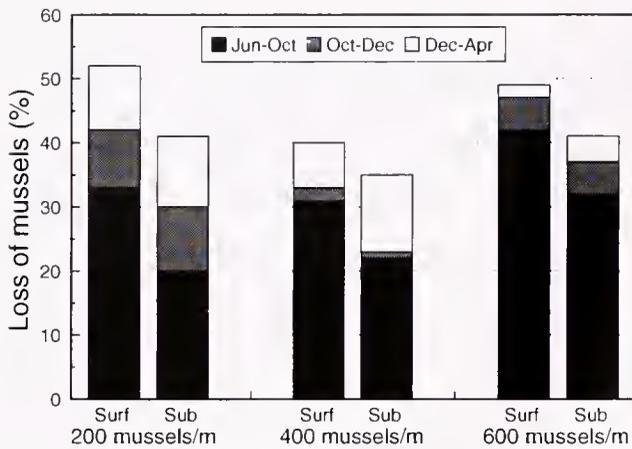


Figure 2. Percentage of mussels lost per sleeve for each position and density, averaged over site. "Surf" and "Sub" refer to the surface and subsurface lines, respectively.

Those treatments which had a significant effect on growth in terms of tissue weight per unit length were site, position, density, and site by position (Table 1). In April, the average tissue weight of a 55-mm mussel was 1.27 g at Lunenburg as compared to 1.46 g at Mahone Bay (Table 2). The decrease in tissue weight at Lunenburg from October to December may be related to fall spawning, as evidenced by the small juveniles (i.e. <5 mm) observed throughout the winter months. During the summer and fall, there was more variation in tissue growth between depths at Mahone Bay than at Lunenburg, probably because of the greater depth range (15 m vs. 2 m). During the winter, Lunenburg exhibited significantly better tissue growth on the subsurface, rather than the surface line, possibly due to the lower concentration of juveniles. Increased competition for food at higher seed densities was reflected in lower tissue weights; April estimates varied from an average of 25.8 mg/mm for the low density sleeves to 23.9 mg/mm for the high density sleeves.

#### (b) Volume and Number of Mussels

Temporal changes in the volume of a sleeve are the net result of shell growth, fall-off, mortality, and recruitment. To eliminate the effect of recruitment at Lunenburg, the volume of juveniles, or "second set," was subtracted from the total volume of each sleeve. As it turned out, site did not have a significant effect on volume (Table 1), only position and density were important. The density effect was predictable given that the three seed densities differed in volume at the beginning of the study. At the final harvest, sleeves suspended from the subsurface lines had a higher

average volume (18.8 l) than those left at the surface (14.5 l) (Table 3). Average sleeve volumes for the three seed densities were 9.2 l (low), 19.1 l (medium), and 21.7 l (high). The minimal increase in volume from October to December may have been related to the low shell growth during this period (Table 2).

The higher volumes obtained from the subsurface sleeves resulted not only from the mussels' greater shell length (Table 2), but more importantly from the higher numbers of mussels per sleeve. Final estimates of mussel density in April ranged from a minimum of 109 mussels/m for the low density sleeves at the surface to a maximum of 336 mussels/m for the high density sleeve at depth. Note that the medium density sleeves generally exhibited lower losses than the other two densities (Fig. 2). The proportion of mussels lost varied according to the sampling period. The highest losses were observed during the summer period; approximately 25% of the mussels on the subsurface lines and 35% of the mussels on the surface lines. Subsequent losses from October to April showed little variation with depth; approximately 10 to 20% depending on the seed density.

#### (c) Secondary Settlement

Secondary settlement of juveniles onto adult mussels was not observed at Mahone Bay, but was very heavy at Lunenburg. The first settlement likely occurred in mid-to-late July, although the presence of very small spat in December and April suggests that larvae may have continued to settle throughout the fall and winter. Those treatments which had a significant effect on the volume of second set were site, position, density, sleeving material, and material by density. In April, the ratio of juveniles to adults averaged 0.49 or 490 ml of juveniles for every liter of adults on the subsurface line versus 0.83 or 830 ml of juveniles for every liter of adults on the surface line (Table 4). This was equivalent to 4.3 l of juveniles per sleeve at the surface versus 3.6 l of juveniles per sleeve at depth. There was also a negative correlation between volume of second set and seed density; in April, the ratio of juveniles to adults was 1.19 for the low density sleeves (5.8 l/sleeve), 0.48 for the medium density sleeves (3.2 l/sleeve), and 0.33 for the high density sleeves (2.4 l/sleeve). Although the analysis indicated that sleeving material, and material by density were significant, these results were confounded by the lack of high density sleeves in the Fresh Klean material; final mean values (Juv/Ad) for the three sleeving materials were 0.65 for Dupont, 0.61 for Italian and 0.74 for Fresh Klean.

#### (d) Production and Yield at Harvest

Those treatments which had a significant effect on production were site, position, density, and site by position (Table 5). In general, the highest production values (g/sleeve/day) were ob-

TABLE 4.

Ratio of juvenile mussels (secondary settlement) to adult mussels (Juv/Ad) for the three seed densities on the surface and subsurface lines at Lunenburg. The values represent the volume (in liters) of juveniles relative to the volume of adults. All densities are in mussels/m of sleeve.

Density	Surface			Subsurface		
	200	400	600	200	400	600
Date	Juv/Ad	Juv/Ad	Juv/Ad	Juv/Ad	Juv/Ad	Juv/Ad
Oct 88	0.34	0.17	0.08	0.24	0.11	0.06
Dec 88	1.19	0.66	0.37	0.72	0.34	0.21
Apr 89	1.54	0.52	0.44	0.84	0.43	0.21

TABLE 5.

Repeated measures analysis of variance of calculated production (kg/sleeve), observed yield (kg/sleeve), and return per sleeve to test for the effects of various treatments.

Source	DF	Production (kg/sleeve)	Yield (kg/sleeve)	Return Per Sleeve
		Mean Square ( $\times 10^6$ )	Mean Square ( $\times 10^4$ )	Mean Square ( $\times 10^4$ )
Site	1	10.5*	29.0*	7.7*
Position	1	34.8*	43.7*	15.1*
Density	2	243.2*	326.0*	16.8*
Sleeving material	2	2.6	3.1	10.8*
Sticks	1	0.1	0.6	13.3*
Site $\times$ position	1	7.9*	7.4	2.6
Material $\times$ density	3	1.5	8.3*	3.5*
Material $\times$ sticks	2	1.1	0.2	0.9
Density $\times$ sticks	2	0.6	0.6	0.7
Error	45	63.6	2.5	1.1

MS refers to the mean square value and DF denotes degrees of freedom. \* indicates a significant effect at  $p < 0.05$ .

served in the summer months and the lowest between October and December (Table 6). A few production values were negative; these may be related to decreases in tissue weight due to spawning (Table 2), or to low growth in conjunction with high losses. The sleeves with the highest overall production values were those grown on the subsurface line at Lunenburg.

Those treatments which affected the final yield were site, position, density and sleeving material by density (Table 5). As noted earlier, the significant density and material by density effect resulted from the experimental design. On average, sleeves grown at depth had a higher yield (9.6 kg) than those held at the surface (7.9 kg), and sleeves from Lunenburg had a higher yield (9.5 kg) than those from Mahone Bay (8.0 kg) (Fig. 3). The highest seed density generated sleeves weighing an average of 12.5 kg as compared to 4.3 kg for sleeves with the lowest seed density. At the final harvest in April, not all the mussels included in the estimates of yield were of market size; 81% had a shell length  $\geq 51$  mm (market size), 70% had a shell length  $> 53$  mm and 59% had a

shell length  $> 55$  mm. Postponing the final harvesting until June 1989 would have increased the proportion of market-size mussels.

#### Economic Assessment of the Grow-out Strategies

##### (a) Set-Up Costs

The seed volume required to obtain a given seed density depends on the size of the seed (i.e. the larger the seed, the greater the volume required). In this study, the cost of a 70-l tub of seed was \$15, but prices may range from \$10 to \$20 depending on the year and the producer. The volumes of seed required varied from approximately 3 l for a low density sleeve to 9 l for a high density sleeve (Table 7). At a price of \$0.21/l, seed costs varied from \$0.63/sleeve for the lowest density to \$1.89/sleeve for the highest density. Note that the average seed size used in this study was quite large, around 30 mm, as compared to a typical sleeving size of 20 to 25 mm; seed costs would have been somewhat lower if smaller seed had been available.

TABLE 6.

Least square means (X) and standard error (SE) of production (g/sleeve/day) over each sampling period.

	Lunenburg				Mahone Bay			
	Surface		Subsurface		Surface		Subsurface	
	X	SE	X	SE	X	SE	X	SE
200 mussels/m								
Jun-Oct	17.0	2.8	23.3	2.8	14.2	2.8	18.8	2.8
Oct-Dec	5.4	8.4	-4.4	8.4	10.4	8.4	8.0	8.4
Dec-Apr	10.6	3.6	16.1	3.6	15.4	3.6	15.2	3.6
400 mussels/m								
Jun-Oct	302	2.8	40.0	2.8	29.2	2.8	34.9	2.8
Oct-Dec	12.7	8.4	-8.8	8.4	8.4	8.4	3.3	8.4
Dec-Apr	22.2	3.6	40.7	3.6	35.2	3.6	39.8	3.6
600 mussels/m								
Jun-Oct	56.5	3.4	57.0	3.4	34.2	3.4	49.6	3.4
Oct-Dec	12.9	10.3	35.0	10.3	25.1	10.3	19.9	10.3
Dec-Apr	25.5	4.4	45.8	4.4	33.8	4.4	30.0	4.4

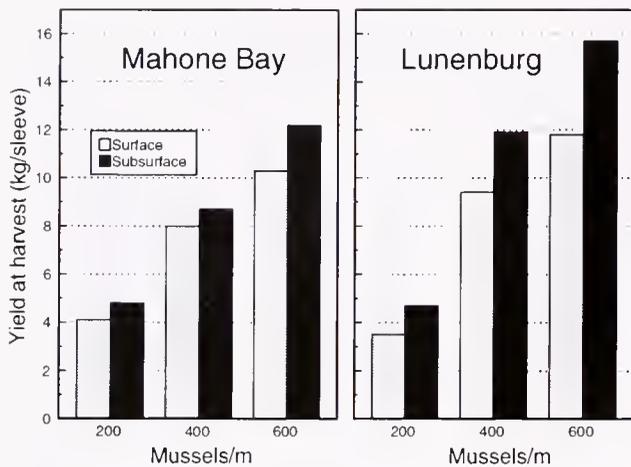


Figure 3. Final yield (kg/sleeve) of mussels at each site, position, and seed density.

Three examples of the seed and equipment costs involved in making up 150 sleeves or the equivalent of one longline were included in Table 7. Note that the cost of the other sleeve types can be determined from the data in this table. Values ranged from a minimum of \$175/line for the low density sleeves of Italian mesh to a maximum of \$378/line for high density sleeves of Dupont mesh. The Fresh Klean material was considerably more expensive than the others, but it was too narrow to hold the highest density of 600 mussels/m. Labour costs were not included as they were effectively the same for all the sleeve types.

#### (b) Economic Return per Sleeve

The various grow-out strategies were ranked by comparing the market value of a particular sleeve type at harvest (e.g. a medium density sleeve with Italian mesh and sticks, grown at depth), with the cost of setting up that sleeve. Note that this approach provided only a relative index of economic return, and was not meant to generate "realistic" estimates of profit per dollar invested. Such estimates could only be determined by incorporating all the costs involved in running a mussel operation.

All five treatments, site, position, density, sleeving material, and sticks, had a significant impact on the ranking of the various strategies (Table 5). Site and position influenced the economic return per sleeve through their effects on yield, whereas density influenced both yield and set-up costs. Sleeving material and sticks affected only the set-up costs; the cheaper the materials, the higher the return. The cheapest combination used in this study was Italian sleeving without sticks.

The effects of site, position and density on the economic return per sleeve are illustrated in Figure 4; values ranged from a minimum of 3.3 for the low density sleeves on the surface line at Lunenburg to a maximum of 6.9 for the medium and high density sleeves on the subsurface line at Lunenburg. Overall, the sleeves from Lunenburg showed a better return (5.4) than those from Mahone Bay (4.6), and those from the subsurface lines gave a better return (5.5) than those grown at the surface (4.5). The medium and high density sleeves gave similar estimates of return (5.5), but the value for low density sleeves was substantially less (3.9).

## DISCUSSION

The yield of mussels per sleeve was significantly influenced by their position in the water column; growing mussels on subsurface, rather than surface lines, translated into an additional 1.7 kg sleeve, or \$280 per line. This higher yield per sleeve was related to various factors: greater shell weight per unit length, lower losses from fall-off and/or mortality, and lower secondary settlement. The most important of these was likely the lower losses experienced by the subsurface sleeves. Higher losses from the surface sleeves were probably related to greater wave action, or to problems associated with higher water temperatures, such as weakening of the byssal threads or increased mortality (Mallet and Carver, in prep.). Natural mortality must have accounted for part of the 40 to 50% loss rate; the expected mortality for mussels of this age and stock is roughly 30% per year (Mallet and Carver, unpubl. data). In addition to experiencing higher losses, the sleeves grown at the surface at Lunenburg had a higher ratio of juveniles to adults, which may account for their substantially lower shell and tissue growth during the winter months. Overall, there was no question that growing mussels on subsurface lines generated a greater economic return than growing mussels at the surface.

At the end of the ten-month production period, the two experimental sites yielded similar numbers of market-size mussels. Slight differences in shell and tissue weight, however, were sufficient to cause significant variations in return between these sites. Previous studies have demonstrated the importance of site in explaining variations in shell and tissue growth (Dickie et al. 1984, Mallet and Carver 1989). In general, Lunenburg showed greater growth in the summer whereas Mahone Bay showed better growth in the fall and winter (see also Mallet and Carver 1989). The relatively poor winter growth at Lunenburg may have been related to the lower food levels associated with ice coverage (Mallet et al. 1986), or competition from the heavy set of juveniles.

In this study, yield per sleeve increased with seed density up to the maximum experimental value of 600 mussels/m. Higher den-

TABLE 7.

Three examples of the initial cost of 150 sleeves (1 line) assuming a typical seed price of \$0.21/l.

Sleeving Material	Seed Density (mussels/m)	Seed Volume (l/sleeve)	Seed Cost (\$/sleeve)	Seed Cost (\$/line)	Sleeving Material (\$/line)	Total Cost (\$/line)
Fresh Klean	200	3.0	0.63	95	87	182
Dupont	400	6.0	1.26	189	38	227
Italian	600	9.0	1.89	284	24	308

Total cost per line is the sum of the seed and the sleeving material. Fresh Klean mesh was \$0.58/sleeve, Dupont was \$0.26/sleeve and Italian was \$0.16/sleeve. If necessary, the respective cost of each of the sleeve types can be calculated from these data. Note that the use of sticks would increase the equipment cost by an additional \$45 per line (\$0.10/stick × 3 sticks/sleeve).

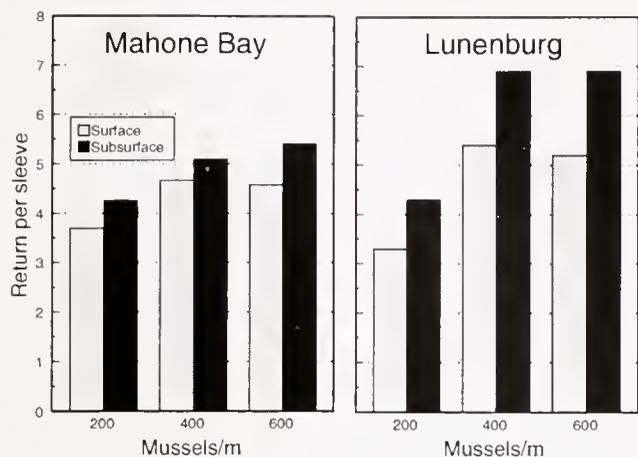


Figure 4. Economic return per sleeve (ratio of revenue obtained vs. initial cost) for each site, position, and seed density.

sities (e.g. 800 mussels/m) were not tested because the sleeving material was too narrow. It is likely, however, that a density of 800 mussels/m or greater would have translated into higher levels of fall-off; estimates of percent loss per sleeve were higher for sleeves with 600 mussels/m than those with 400 mussels/m (Fig. 2). Another problem associated with higher seed densities is the tendency of mussels to become jammed in the center of the sleeve where they die from lack of food or oxygen. Competition for food also tends to increase at higher densities; in this study, increases in seed density did not affect shell growth, but did have a negative impact on tissue weight. It is interesting to note that in 1988 most producers typically aimed for a sleeving density of 525 mussels/m, but over the last few years the tendency has been to reduce this density, in some cases to as low as 450 mussels/m (Mallet and Carver, pers. obs.). On the other hand, this study also indicated that sleeves with higher seed densities experienced relatively less juvenile settlement. One possibility is that dense aggregations of adult mussels can reduce the number of potential settlers through filtration (Mileikovsky 1974). Another suggestion is that the greater accumulation of biodeposits on the denser sleeves leaves relatively less surface area for larval attachment.

The sleeving materials tested in this study had no effect on mussel weight or volume of mussels retained. It has been suggested that the woven design of the Fresh Klean material would

reduce seed losses during the process of sleeving and attachment of the sleeves to the longlines. Our observations suggest, however, that this advantage may be offset by the tendency of mussels to remain trapped in the center of the sleeve, resulting in higher mortalities. Overall, it was concluded that the cheapest material, in this case the Italian mesh, gave the best return per sleeve. The performance of the Dupont material was similar to that of the Italian, but its greater cost reduced its attractiveness.

This study did not justify the use of sticks as a means of increasing yield per sleeve; at both sites the yields were similar with or without sticks. In 1988 the use of sticks was a widely used strategy in Prince Edward Island where severe levels of fall-off are often associated with weak byssal attachment during the warmer summer months (Mallet and Carver, pers. obs.). Since then, however, the popularity of this technique has declined, possibly because of the costs involved, or the development of more effective methods.

At the end of this study, the various grow-out strategies were ranked according to their economic return. Note, however, that these results would need to be re-considered before extrapolating to a full-scale commercial operation. For example, although the medium and high densities (400 and 600 mussels/m) showed a similar return per sleeve (revenue per dollar invested), the latter density generated a higher absolute profit per sleeve (i.e. revenue minus investment). In cases where the aim is to maximize the net profit from a given line, it may be advisable to weigh the relative advantages of using a higher seed density against the additional cost of floatation to support the greater weight.

Several results from this study are worth noting: (1) maintaining sleeves on subsurface lines significantly increased the yield at harvest; furthermore, this strategy reduced the probability of conflict with other users of coastal waters; (2) the choice of sleeving material did not affect yield; hence, the cheaper the material the greater the return; and, (3) differences between sites influenced the economic return per sleeve.

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**PROCEEDINGS OF THE SPECIAL SYMPOSIUM: SHELL DISEASE IN  
MARINE CRUSTACEANS**

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## INTRODUCTION TO A SYMPOSIUM ON SHELL DISEASE IN MARINE CRUSTACEANS

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Ninety years ago a new disease of Baltic crustaceans was described (Happich, 1900)—a disease of bacterial etiology that caused severe exoskeletal erosion. Since then, down through the decades and in many places in the world, more than 100 papers have been published on this condition, now called "shell disease" or "chitinoeciasis." Indications that environmental stress, particularly that resulting from estuarine/coastal pollution, may influence the prevalence and severity of the disease have prompted a renewed examination of the disease process, and the factors, internal and external, that may influence it.

Early in 1989, a group of specialists interested in shell disease assembled at NOAA's Sandy Hook (NJ) Laboratory to examine all available information about shell disease and its possible relation-

ship to pollution. The report of that working group was published late in 1989 (Sindermann et al. 1989), implicating environmental stress, but also describing a much broader resource concern resulting from the shell disease syndrome.

In accord with the perceived broader scope of the shell disease problem, some of the members of the original working group, and other scientists, were invited to prepare and contribute papers for this symposium, which reviews the status of shell disease as it occurs on the Atlantic coast of United States. These papers have been assembled in this volume of the *Journal of Shellfish Research* to provide a readily accessible source of detailed information relevant to the shell disease syndrome in crustacean resource species of the Atlantic coast.

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# SHELL DISEASE IN AMERICAN LOBSTER (*HOMARUS AMERICANUS*, H. MILNE EDWARDS, 1837) FROM MASSACHUSETTS COASTAL WATERS WITH CONSIDERATIONS FOR STANDARDIZING SAMPLING

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**ABSTRACT** Shell disease in the American lobster (*Homarus americanus*) from six coastal Massachusetts sites was investigated. A total 4,791 lobster was collected via a commercial lobster sea sampling program during May through November, 1989. The effect of a number of variables on shell disease prevalence was evaluated in order to standardize assessments of the condition.

Disease signs were not uniformly distributed throughout the length range of the samples. Prevalence was highest and severity greatest in the larger size groups indicating an inverse relationship with molt frequency. The correlation was significant for hard-shelled lobster but not for new-shelled lobster. Significant differences in disease prevalence were observed between the sexes. This was primarily due to the high level of disease observed among ovigerous females. However, mature non-ovigerous females also exhibited a significantly greater frequency of disease signs than males.

These factors are important considerations in comparative analyses. Samples used in trend studies should be standardized according to size, sex, ovigerous and molt condition, and severity.

**KEY WORDS:** shell disease, American lobster, *Homarus americanus*, variability, standardized sampling

## INTRODUCTION

Shell disease is a commonly occurring disease of marine and fresh water crustaceans. It is characterized by a deterioration of the chitinous exoskeleton by chitinoclastic (chitin-consuming) microorganisms which gradually erode and pit the shell, and in advanced cases uncover the epithelium, and create necrotic lesions (Malloy 1978, Sindermann 1970, Rosen 1970, Dow et al. 1975, Stewart 1980). Bacteria and fungi have been implicated; however, several species of the bacterial genera, *Vibrio*, *Aeromonas*, and *Pseudomonas*, are most often cited as causative agents (Getchell 1989, Sindermann 1989).

This disease can affect the marketability of crustacean species by creating an unsightly appearance, weakness, and elevated mortality. Consequently, shell disease monitoring was incorporated into our ongoing Massachusetts lobster stock assessment program via commercial sea sampling. The intent was to define the variables which affect shell disease prevalence and thereby to standardize estimates of it.

## METHODS

Commercial lobster sea sampling is conducted annually in six coastal Massachusetts regions (Fig. 1) from May through November to generate catch-per unit-effort (CPUE) and biological statistics for stock assessments. Traps are sampled twice per month in each region. In 1989, the disease monitoring segment of the program was modified by incorporating a subsampling technique in which approximately 50 lobsters were sampled per trip. The last trawl (or two depending upon lobster density) hauled per day was sampled for shell disease and associated biological data only. This allowed adequate time to discern shell disease signs and thereby improve data quality.

Standardization of lobster shell disease sampling and evaluation was attempted to allow comparative or trend analyses. The variables of lobster size, sex, condition, including molt stage and presence of eggs (brown = developing, green = newly extruded), severity of disease signs, and anatomical location were noted.

Shell disease signs were categorized as *minor pitting*: single or multiple pits or infected pores with localized shell discoloration (dark brown or black) and deterioration; *erosion*: merging or connecting of localized pits and tunneling via chitinolytic activity; or

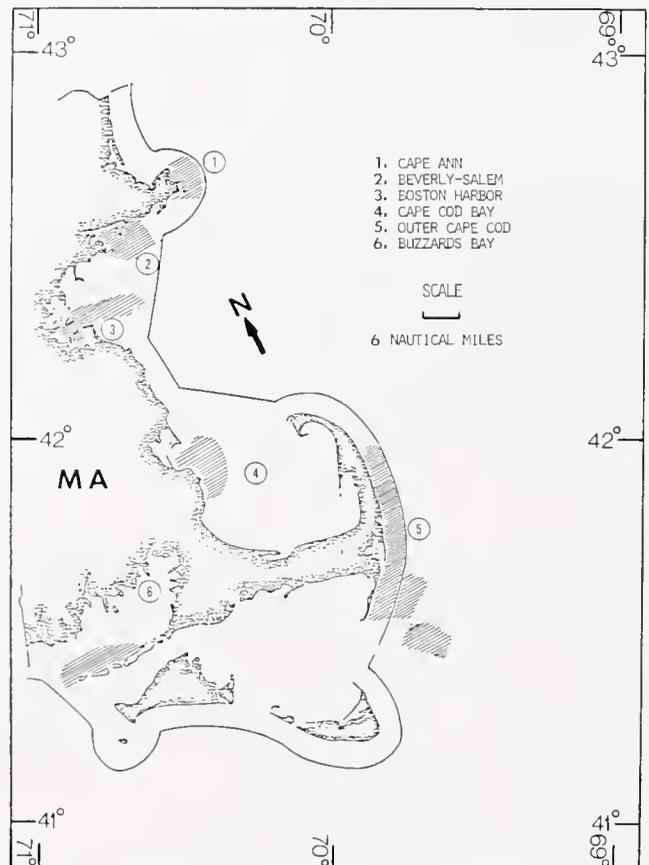


Figure 1. Map of Massachusetts coast showing regions and Massachusetts territorial line. Hatch-marked areas represent locations of gear sampled.

TABLE 1.

Pearson's *r* correlation matrix for variables tested in association with shell disease prevalence of American lobster, Massachusetts coastal waters, 1989.

	Shell Disease (SD)	Carapace Length (CL)	Shell Hardness (SH)	Sex & Ovig. Condition (SO)	Anatomical Location (AL)	Region (R)	Month (M)
SD	1.0000						
CL	0.0531**	1.0000					
SH	0.2052**	-0.0349	1.0000				
SO	-0.2542**	-0.1073**	-0.1215**	1.0000			
AL	0.7086**	0.0418*	0.1495**	-0.1485**	1.0000		
R	0.2775**	0.0966**	-0.0482**	-0.1702**	0.1700**	1.0000	
M	0.0937**	0.0791**	-0.1851**	-0.0318	0.0615**	-0.0548**	1.0000

\* =  $P \leq 0.01$ ; \*\* =  $P \leq 0.001$

*ulceration*: when extensive erosion has destroyed the chitinous layers and uncovered underlying tissue, resulting in secondary infection.

Pearson's *r* correlation coefficients were computed to determine the interdependence of variables. Duncan's multiple range test (Steele and Torrie 1960) was used to determine pairs of stations significantly different at the 0.05 alpha level. The Mann-Whitney U-Wilcoxon Rank Sum W two-sample test (Sokal and Rohlf 1969) was applied to examine the effects of variables on disease incidence.

A total 4,791 lobster was sampled for shell disease through commercial sea sampling off Massachusetts in 1989.

## RESULTS

Shell disease prevalence was correlated with all parameters tested including carapace length (CL), shell hardness, sex and ovigerous condition, anatomical location of disease signs, region and month (Table 1). Many of these "independent" variables were also intercorrelated due to their seasonal (monthly) and regional variability.

The coastwide mean CL of lobster sampled was significantly greater during September through November than in earlier months ( $P = 0.05$ ; Table 2). Lobster from the outer Cape Cod region averaged 92.9 mm CL. This was significantly larger than

TABLE 2.

Mean carapace length (mm) of American lobster by month with pairs of months significantly different at  $\alpha = 0.05$  (\*), Massachusetts coastal waters, 1989.

Mean	Month	Month						
		6	5	8	7	9	10	11
81.3025	6							
81.3064	5							
81.3556	8							
81.8947	7							
82.6071	9	*	*	*				
82.7938	10	*	*	*				
84.0371	11	*	*	*	*	*	*	

the other regions which ranged from 78.6 to 82.1 mm CL. Although samples from the latter regions exhibited similar means, significant differences were found among them (Table 3). Mean sizes of lobster sampled by sex and condition (shell hardness, ovigerous) within each region also exhibited variability (Table 4) which warranted standardization in disease analyses.

The positive correlation between shell disease and lobster size (Table 1) was strong for hard-shelled lobster ( $r = 0.0679$ ,  $P \leq 0.001$ ; Fig. 2) and weak for new-shelled lobster ( $r = -0.0144$ ,  $P = 0.692$ ; Fig. 3). Subsequent analyses were conducted on only hard-shelled lobster in the 71-90 mm CL range. This size range is most often available to commercial traps and represents the bulk of the samples collected.

Analyses by region indicated that Buzzards Bay lobster exhibited more shell disease than other regions (Table 5). The coastwide percent of females infected (including ovigerous lobster) was higher than males ( $P < 0.0001$ , Fig. 4). Most regional female indices were generally higher than males except in Beverly-Salem where no egg-bearing females were sampled. Non-ovigerous females also exhibited a disease prevalence which was higher than that of males but not significantly different ( $P = 0.0978$ ). However, an analysis by size indicated that the percentages of shell disease for non-ovigerous females and males smaller than 81 mm CL were not significantly different ( $P = 0.2638$ ) while those for lobster greater than or equal to 81 mm CL were significantly different ( $P = 0.0331$ ).

Ovigerous (egg-bearing) females in the 71-90 mm CL category

TABLE 3.

Mean carapace length (mm) of American lobster by region with pairs of regions significantly different of  $\alpha = 0.05$  (\*), Massachusetts coastal waters, 1989.

Mean	Region	Region					
		2	4	6	3	1	5
78.5666	Beverly-Salem (2)						
79.8237	Cape Cod Bay (4)	*					
80.0791	Buzzards Bay (6)	*					
80.6257	Boston Harbor (3)	*	*				
82.1091	Cape Ann (1)	*	*	*	*		
97.9165	Outer Cape Cod (5)	*	*	*	*	*	

TABLE 4.

Mean carapace lengths of American lobster by sex and ovigerous condition for all lobster, hard-shelled, and new-shelled lobster sampled for shell disease Massachusetts coastal waters, 1989.

	Males	Females	Nonovig. Females	Ovigerous Females
All Lobster	82.0	82.3	81.1	89.1
Cape Ann	82.0	82.2	81.7	95.8
Beverly-Salem	78.5	78.6	78.6	—
Boston Harbor	81.5	79.9	79.7	86.6
Cape Cod Bay	79.9	79.7	79.4	87.5
Outer Cape Cod	95.9	99.5	94.5	107.1
Buzzards Bay	79.4	80.4	79.9	81.4
Hard-Shelled Lobster	81.6	82.3	80.9	89.1
Cape Ann	82.0	82.2	81.7	95.8
Beverly-Salem	78.0	78.5	78.5	—
Boston Harbor	78.4	78.9	78.4	86.6
Cape Cod Bay	79.6	79.5	79.2	87.5
Outer Cape Cod	96.0	99.5	94.0	107.1
Buzzards Bay	79.3	80.3	79.6	81.4
New-Shelled Lobster	83.8	82.1	82.1	—
Cape Ann	90.0	—	—	—
Beverly-Salem	86.1	84.3	84.3	—
Boston Harbor	83.7	81.1	81.1	—
Cape Cod Bay	82.8	83.3	83.3	—
Outer Cape Cod	94.6	99.1	99.1	—
Buzzards Bay	79.5	81.1	81.1	—

exhibited a greater prevalence of disease signs, 83.9%, than similar sized non-ovigerous females, 29.4% ( $P < 0.0001$ ) or males 26.6% ( $P < 0.0001$ ). Although brown ovigerous females had more disease signs than green ovigerous females (Fig. 5), the difference was not significant ( $P = 0.2570$ ).

An analysis of shell disease in hardshelled lobster by 10 mm groupings and severity indicated that ulceration was not prevalent until larger size groups (Fig. 6). Consequently, it was observed at a greater frequency in the outer Cape Cod (1.1%) and Cape Ann (2.0%) data sets where larger lobster (>110 mm) predominated (0.0–0.8% in other inshore regions).

Analyses of hardshelled lobster by severity and anatomical lo-

cation revealed that most shell disease was found on claws (generally the ventral side) followed by the tail, carapace, and legs (Fig. 7).

## DISCUSSION

An attempt was made to standardize lobster shell disease prevalence data for comparative analyses by assessing the effect on it of a number of variables. Lobster size, sex, and ovigerous condition exhibited the greatest influence on shell disease prevalence.

The increase in disease prevalence with lobster size indicates a relationship with environmental exposure time. Lobster which measure from 71–98 mm carapace length may molt 0–2 times

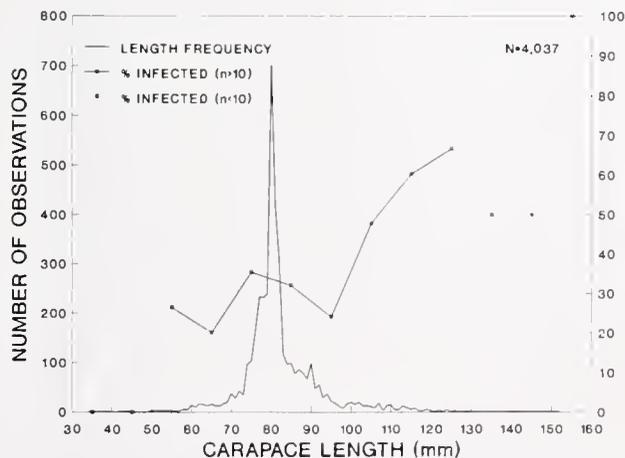


Figure 2. Relationship between shell disease in hard-shelled American lobster and lobster carapace length plotted against length frequency of samples, Massachusetts coastal waters, 1989.

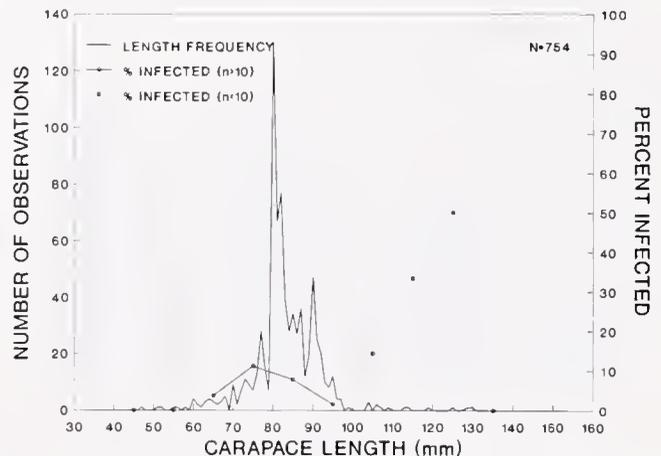


Figure 3. Relationship between shell disease in new-shelled American lobster and lobster carapace length, plotted against length frequency of samples, Massachusetts coastal waters, 1989.

TABLE 5.

Shell disease (% infected) in hard-shell American lobster (71–90 mm CL) from six Massachusetts coastal regions, 1989. Sample sizes are in parentheses.

	ALL	Males	Females	Ovigerous Females	Nonovigerous Females
Regions Combined	33.3 (3316)	26.6 (1246)	37.2 (2070)	29.4 (1772)	83.9 (298)
Cape Ann	11.3 (886)	7.6 (344)	13.7 (542)	13.0 (530)	41.7 (12)
Beverly-Salem	29.2 (391)	29.2 (178)	29.1 (213)	29.1 (213)	—
Boston Harbor	32.9 (359)	23.8 (126)	37.8 (233)	335.9 (220)	69.2 (13)
Cape Cod Bay	42.8 (668)	36.6 (254)	46.6 (414)	45.0 (400)	92.9 (14)
Outer Cape Cod	26.7 (131)	18.6 (59)	33.3 (72)	27.7 (65)	85.7 (7)
Buzzards Bay	51.1 (881)	42.1 (285)	55.4 (596)	32.8 (344)	86.1 (252)

annually (Hughes and Matthiessen 1962). Smaller lobster molt with greater frequency and are more likely to shed their shells before chitin deterioration becomes severe enough to effect necrosis of underlying tissue. Hard-shelled lobster had a higher infection rate than new-shelled lobster because their shells are exposed to the environment for a longer period of time. Ovigerous females exhibited the greatest prevalence of shell disease because molting is delayed until after hatching of their eggs and their shells are thus retained for a longer period. However, non-ovigerous females also generally had more disease signs than males. Some spent egg-bearing females may have been included in the samples analyzed but the comparatively slower growth of females when sexually mature, i.e. reduced molt frequency from the 2-year ovarian cycle, is likely responsible. This is emphasized by the significantly greater disease prevalence for non-ovigerous females which were larger than 80 mm CL. Estrella and McKiernan (1989) defined the size at 50% maturity for female American lobster in Massachusetts coastal waters as 76 mm CL in Buzzards Bay, 87 mm CL in Cape Cod Bay and Boston Harbor, 90 mm CL off Cape Ann, and 97 mm CL off outer Cape Cod.

The large number of specimens with signs of pitting, i.e. infected shell pores, affirms the ever-present availability of the causative agent(s) and the need to screen samples adequately to develop accurate disease indices. The greater prevalence of ulcer-

ation in Cape Ann and outer Cape Cod regions was due to the larger sized (older) lobster there and their comparatively longer shell exposure times.

Green ovigerous females have a shorter exposure time than brown ovigerous females. They may extrude eggs from several to 10 months after molting whereas brown ovigerous females may have gone up to an additional year longer than that period before egg hatching and subsequent molting. The lack of a statistically significant difference in shell disease prevalence between green and brown ovigerous females may be due to the lack of fall samples of brown ovigerous females and the lack of spring samples of green ovigerous females (Fig. 5).

The high frequency of shell disease signs on the ventral surface of the major claws may be the result of regular contact of this anatomical area with the sediment. Abrasion of the shell pores there probably enhances infection. Young and Pearce (1975) made similar observations on lobster and rock crabs (*Cancer irroratus*) in heavily polluted areas of the New York Bight. They stated that the tips of the walking legs were often affected.

The methodology described was developed as a standard approach to monitoring trends in shell disease prevalence in Massachusetts coastal waters. Accordingly, the comparability of these data to previous studies is not possible due to differences among them in lobster size distribution, molt stage, sex ratio, ovigerous condition, sampling location, and respective sample sizes. Also,

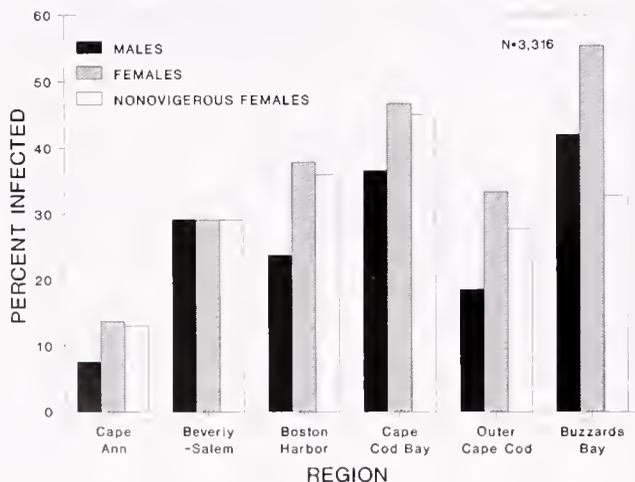


Figure 4. Shell disease in hard-shelled American lobster (71–90 mm CL) by sex and ovigerous condition from six Massachusetts coastal regions, 1989.

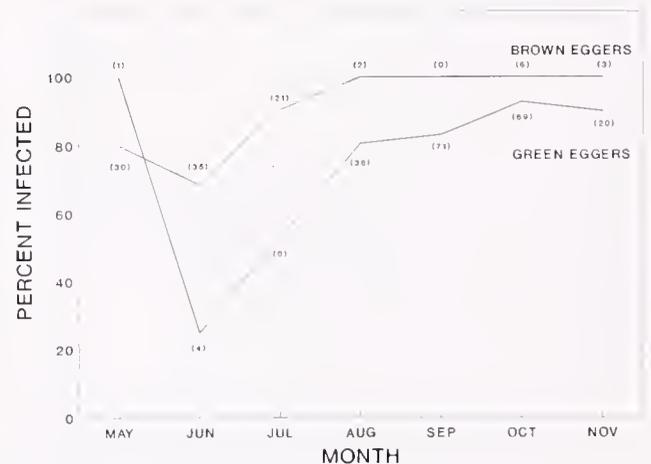


Figure 5. Shell disease in brown (developing eggs) and green (newly extruded eggs) egg-bearing American lobster females by month, Massachusetts coastal waters 1989.

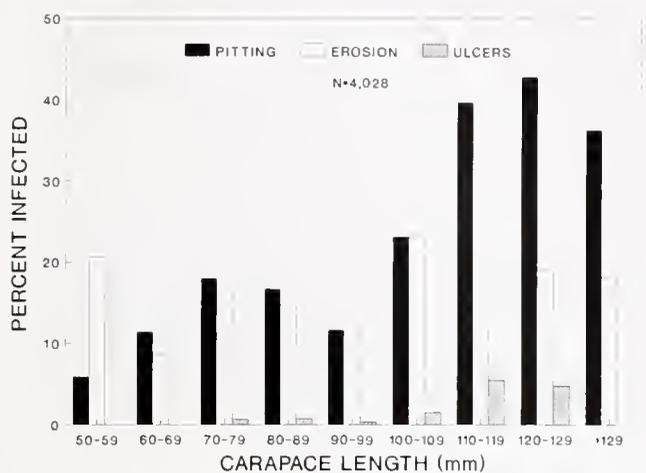


Figure 6. Shell disease in hard-shelled American lobster by 10 mm CL groupings and severity, Massachusetts coastal waters, 1989.

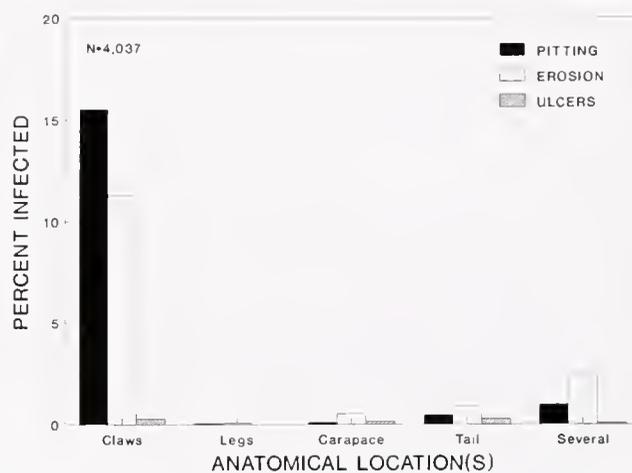


Figure 7. Shell disease in hard-shelled American lobster by severity and anatomical location, Massachusetts coastal waters, 1989.

the inclusion of disease signs at the pore level in gross evaluations has raised prevalence estimates from those previously calculated and warrants reinitiation of data time series.

Standardization should improve prevalence estimates relative to concerns about environmental conditions which can cause stress in crustacea and enhance shell disease. Although the signs of shell erosion and melanization of diseased tissue in crustaceans are ubiquitous, high prevalences have been reported near ocean disposal sites (Gopalan and Young 1975, Young and Pearce 1975, Bodammer and Sawyer 1981, Sawyer 1982, Sawyer et al. 1983). In 1988–1989, a joint NOAA/EPA working group made a detailed examination of available information on shell disease in crustaceans from the New York Bight and elsewhere (Sindermann et al. 1989) and concluded that evidence exists for an association of shell disease with habitat degradation.

Previous observations of shell disease in Massachusetts coastal waters (Estrella 1984) indicated an elevated prevalence in Buzzards Bay. Although the data are not directly comparable, this observation is consistent with results of the present study.

Environmental conditions may be responsible. Periodic outbreaks of shell disease in Nova Scotian and Maine coastal impoundments have historically affected lobsters imported into Massachusetts and other states. Complaints have been voiced by local lobster dealers of unaesthetic appearance, weakness, and enhanced mortality among these imports. Heavy organic loading and poor water quality in impoundments, which allow bacteria to flourish, appear to be responsible. Similar conditions have occurred in the wild as a result of marine disposal practices. Sindermann (1989) and Sindermann et al. (1989) summarize published accounts of shell disease prevalence in the vicinity of degraded habitats.

The water exchange rate and turbidity at sampling sites are possible factors affecting disease incidence, Buzzards Bay is a comparatively closed, shallow embayment exhibiting poor circulation (Gilbert et al. 1973) and subsequently warmer bottom tem-

peratures during summer months (Colton and Stoddard 1973). These conditions may promote and maintain bacterial growth. Buzzards Bay waters consistently contain high levels of dissolved solids (Gilbert et al. 1973). Stormy conditions apparently cause resuspension of bottom sediments. In contrast, other regions sampled, with the exception of Boston Harbor, are generally characterized by relatively open water with a greater depth range and cooler water temperatures.

Nevertheless, establishing cause and effect is complicated by the potential stress from industrial contaminants such as PCB's, heavy metals, and hydrocarbons which have been found throughout Buzzards Bay (Gilbert et al. 1973) with the highest levels observed in the New Bedford Harbor region (Ellis et al., unpublished manuscript 1977, Kolek and Ceurvels 1981, Weaver 1982). The New Bedford Harbor area is also heavily polluted with domestic sewage (Massachusetts Department of Environmental Quality and Engineering, unpublished laboratory results, 1983). However, such pollutants are not limited to this area of Massachusetts coastal waters and occur throughout Massachusetts Bay and Cape Cod Bay (Gilbert et al. 1976, Boehm 1984).

Such conditions provide extensive opportunity for future study of the effects of pollutants. A standardized approach to data acquisition and analysis, as outlined above, should be helpful in disease assessments.

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## LOBSTER SHELL DISEASE SURVEY

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**ABSTRACT** The prevalence of shell disease among lobsters held in confinement was examined within the Maine Lobster industry. Retail dealers and impoundment owners handling large volumes of live lobsters were contacted by mail. The twenty-four item questionnaire addressed the impact of lobster shell disease on their operation and the preventive measures they prescribe to control their losses. Follow-up phone interviews were conducted with those respondents whose lobster stocks were most often afflicted with this disease syndrome. The scope of shell disease in Maine's holding facilities has been shown to be a significant problem, particularly for poundkeepers. The prospects for keeping this disease under control depend on how each operator handles and cares for his lobsters.

**KEY WORDS:** shell disease, lobsters, Maine, prevention, dealers

### INTRODUCTION

The recent controversy surrounding uninspected seafood supplies has sensitized both harvesters and wholesalers of lobsters. The need to provide an aesthetically pleasing and healthy product is of utmost importance. The appearance of a lobster with lesions on its shell immediately reduces the value of this crustacean, endangers the remaining stock, and erodes the consumer's confidence in the Maine lobster industry.

To control this disease, proper preventive measure can be adopted (Stewart 1980). Only under degraded or crowded conditions does shell disease prove to be a serious problem. What is the scope of the shell disease problem in Maine's lobster industry? Are Maine's lobster holding facilities employing enough preventive measures to adequately fight this problem?

### APPROACH

During January, 1990, a questionnaire was sent to forty-five members of the Maine Lobster Pound Association and one hundred twenty-five lobster dealers to document the impact of shell disease within Maine's lobster holding facilities. The survey was financed by the Seed Lobster Fund which is administered by the Department of Marine Resources. A series of twenty-four questions was asked covering the storage facility itself, the effect that shell disease has had (if any), and the practical measures that have been employed in preventing or controlling shell disease. Follow-up phone interviews were conducted with those operators most affected by the shell disease problem. As of May 7, 1990, 32 of 45 poundkeepers and 34 of 125 lobster dealers surveyed had replied.

The goal of this initial effort was not to conduct a statistical survey, but to gather information to put the shell disease problem in perspective and subsequently advise the lobster industry on the best approaches to combat this disease. The following information is a summary of the survey data, describing physical features of Maine's lobster pounds and dealerships and how these storage facilities are affected by shell disease. A list of recommended control measures that are currently being practised is also included.

### RESULTS

#### (A) Physical Features and Operational Practices at Maine's Lobster Dealerships and Tidal Impoundments

- Dealers have the capacity to store an average of 10,000# of lobsters in tanks, crates, or cars (ranging from 1500# to 60,000#).
- Pound operators have the capacity to store an average of 117,000# of lobsters in their tidal impoundments (ranging from 35,000# to 350,000#).
- Pound operators commonly stock their pounds at a density of approximately one pound per square foot of surface area. Dealers stock their tanks at a higher, undocumented level.
- Both types of operations subjectively judged their water exchange rates as good or excellent.
- The highest water temperatures range from 50-70°F.
- Both types of facilities are located over or near a variety of bottom types dominated by mud bottoms.
- Sixty percent of dealers remove their lobsters by dipnetting.
- Most poundkeepers harvest their lobsters with the aid of a seine drag, divers, or an airlift.
- The cleaning method employed is most often simple flushing.

#### (B) How Are Maine's Lobster Dealers Affected By Shell Disease?

- Twenty-one percent of dealers find shell disease in 1-5% of their lobsters when first purchased.
- Eighteen percent discover shell disease after a period of storage.
- Those affected by shell disease purchase an average of twenty-four percent of their lobsters from Canada.
- Shell disease is prevalent in winter, spring, and summer.
- Lesions start to appear after 3-4 weeks in storage.
- Lesions most often occur on the upper surfaces of the body followed by the claws.
- Seventy-eight percent of affected dealers cull out infected lobsters prior to storage.
- Most dealers cull at the stage lesions are minor or moderate.
- All dealers checked their lobsters before storage.
- Sixty-seven percent of those affected by shell disease also re-check shell quality during storage.
- Seventy-eight percent of dealers remove mortalities daily.
- Most dealers discard mortalities in refuse containers. Two affected dealers dumped dead lobsters in waters nearby.

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(C) How Are Maine's Lobster Pounds Affected By Shell Disease?

- Sixteen percent of pound owners find shell disease in 1–5% of their lobsters when first purchased and stored.
- Forty percent of pound owners discover shell disease after long term storage.
- Those affected by shell disease purchase twenty-three percent of their lobsters from Canada, while those unaffected pound operators only purchase thirteen percent Canadian product. Shell disease is most prevalent in winter, then present somewhat in spring.
- Fifty percent of affected pound owners observe lesions after four months of storage.
- Lesions appear on the upper surfaces of claws, body, and tail in decreasing order of frequency.
- Sixty-nine percent of affected pound owners cull out infected lobsters prior to storage. Others don't observe lesions initially.
- Most pound owners cull out infected lobsters when lesions are minor or moderate.
- Each lobster is checked before storage.
- Sixty-nine percent of those pound owners affected recheck shell quality at some time during storage.
- Thirty-one percent of pound owners remove mortalities daily. Thirty-four percent remove dead lobsters only at harvest time.
- Most pound owners discard mortalities in refuse containers. Two affected pound keepers piled their dead lobsters on the shore.
- More affected pound keepers (85%) drag their lobsters as a harvest method than pound owners unaffected by shell disease (26%).

(D) List Of Control Measures For Preventing Shell Disease

- 1) Source—Purchase from non-infected areas.
- 2) Inspection—Examine each lobster prior to storage.
- 3) Density—Limit overcrowding.
- 4) Hygiene—Remove mortalities and old shells promptly.

- 5) Husbandry—Provide adequate feed.
- 6) Harvest—Avoid damage by harmful gear.
- 7) Monitor—Recheck quality of exoskeletons periodically.
- 8) Environment—Avoid rapid changes in salinity, temperature, and oxygen levels.

#### DISCUSSION

High prevalences of shell disease occur in captive lobsters when compared to natural populations (Rosen 1970, Johnson 1983). Shell erosion is a particular problem in impoundments, where because of overcrowding, there is a greater likelihood of damage to the cuticle and higher levels of organic matter (Sindermann 1989). Both of these factors encourage bacterial populations that degrade the lobster's shell (Malloy 1978). These chitin-degrading organisms also need the assistance of other environmental stressors such as low oxygen levels, temperature extremes, and high ammonia concentrations (Sindermann et al. 1989). Because chitinolytic bacteria are part of the normal flora of the lobster, they are constantly being introduced into the holding facility. Often, infections may be acquired and develop after impoundment. More detailed study is needed to quantify when the majority of infections are acquired, before or after capture.

Those lobsters that have recently shed are more susceptible to the combined stresses of poor water quality and overcrowding. Their shells may never properly "harden up" and therefore suffer the consequences during a period of prolonged storage. The duration of the impoundment can then be important as each lobster attempts to repair the damage to its exoskeleton.

Presently, Maine's lobster dealers and pound operators are taking into consideration all of the factors discussed here and applying them as preventive measures to control shell disease. Shell disease in Maine's lobster holding facilities has been shown to be a significant problem, particularly for pound keepers. The prospects for keeping this disease under control depend on how each operator handles and cares for his valuable lobsters.

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## SHELL DISEASE IN MARINE CRUSTACEANS—A CONCEPTUAL APPROACH

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**ABSTRACT** Shell disease of marine crustaceans, recognized early in this century as a problem in impounded populations, has been investigated more recently because of its possible association with degraded habitats and because of its potential role in marine aquaculture. Understanding of this microbially induced disease condition has progressed to a point where tentative hypotheses may be proposed: (1) chitin deposition is an important defense mechanism in Crustacea; (2) shell disease is an external indication of metabolic disturbance or trauma, compounded by the activity of chitinoclastic microorganisms; (3) shell disease is intimately associated with success or failure of processes of external defense and wound repair in crustaceans; (4) shell disease may be less important in species with short-life spans than in longer-lived species; (5) shell disease may occur in particularly high prevalences in offshore deepwater crustaceans; (6) pollutants (or other stressors) may foster the development and increase the severity of shell disease; (7) shell disease is a controllable condition in captive or cultured crustacean populations.

Evidence to support these hypotheses varies in "robustness" but a conceptual base for understanding the significance of shell disease in marine crustaceans seems to be emerging.

Recent expansion of interest in shell disease of economically important marine crustaceans—especially lobsters, shrimp, and crabs—has resulted in the publication of many papers which point to the universality of the disease and which suggest severe effects on individual animals (see Getchell 1989 and Sindermann 1989 for references). This paper attempts to integrate, through the development of a series of seven hypotheses (which are not mutually exclusive), the present understanding of shell disease.

The hypotheses are:

- I. Chitin deposition is an important defense mechanism in Crustacea.
- II. Shell disease is an external indication of metabolic disturbance or trauma, compounded by the activity of chitinoclastic microorganisms.
- III. Shell disease is intimately associated with success or failure of processes of external defenses and wound repair in crustaceans.
- IV. Pollutants (or other stressors) may foster the development and increase the severity of shell disease.
- V. Shell disease may be less important in species with short life spans than in longer-lived species.
- VI. Shell disease may occur in high prevalence in offshore deepwater crustaceans.
- VII. Shell disease is a controllable condition in captive or cultured crustacean populations.

The approach used in this paper is to state the hypothesis, then to assemble relevant data of recent origin to explore the validity of the statement.

### *Hypothesis I.*

*Chitin deposition is an important defense mechanism in Crustacea*—maintaining an intact cuticle that is resistant to invasion by many microorganisms. Chitin deposition is most dramatic during the immediate post-molt period or during wound repair, but it may occur as a more-or-less continuous process of secretion by the epidermis through cuticular pores and ducts. The crustacean exoskeleton is considered by some to be a living tissue (Skinner 1962, Roer and Dillaman 1984).

### *Hypothesis II.*

*Shell disease is an external indication of metabolic disturbance or trauma which results in failure of an important defense mechanism—chitin deposition—to keep pace with activities of chitin degrading*

*microorganisms.* Abnormal metabolism may be a consequence of the presence of environmental stressors (pollutant chemicals, anoxia, etc.) or of physiological changes associated with poor nutrition or advancing age (slowing or cessation of molting, for example). The disease may also occur after abrasion or other damage to the exoskeleton, but its genesis in stressed individuals may not be dependent on such damage (Cipriani et al. 1980, Stewart 1984).

### *Hypothesis III.*

*Shell disease is intimately associated with processes involved in external defense and wound repair in crustaceans,* particularly with melanization as a result of phenoloxidase activity (Söderhäll and Unestam 1975, 1979). Melanization of the exoskeleton in the areas of chitinoclastic action is an early and obvious sign of the disease; in species such as the red crab and rock crab, extensive blackening may actually precede disruption of the carapace. Initial response to cuticular disruption is by hemocytes, forming a cellular plug—but this is eventually replaced by a chitinous layer, and by melanin deposition in the traumatized area.

The process of wound repair in the larger crustaceans has been described in several papers, notably those of Fontaine and Lightner (1973, 1976), Unestam and Ajazon (1976) and Söderhäll and Unestam (1979). Since many of the observations of shell disease implicate initial mechanical damage to some or all of the cuticular layers, it seems important to examine simultaneously the constructive processes of wound repair and the destructive processes of shell disease, since the outcome may be critical to survival of the traumatized animal.

A sequence of events seems to emerge from published literature and personal observations—events that may be altered to some extent by the severity of the initiating physical disruption of the cuticle. Considering first the much more frequent examples of minor injuries, in which parts of the epicuticle are destroyed, and some of the underlying chitinous layers are disrupted, the sequence seems to involve initial invasion by bacteria, indicated by a white halo in the cuticle around the site of injury. This is followed within a few days by melanization of the area, producing typical blackened lesions, which may be progressive. Formation of a hemocyte plug at the site of injury has been seen and reported by several authors. The plug is gradually replaced by epithelium, and a new chitinous layer is formed.

In more serious injuries, such as penetrating

wounds which perforate the entire cuticle and disrupt underlying tissue, the process of containment was best described by Fontaine and Lightner (1973, 1975) in the following steps:

- (1) migration of hemocytes to the traumatized area, forming a hemocytic plug, with active phagocytosis of cellular debris, and possibly diapedesis through cuticular pores;
- (2) infiltration of the area beneath the epidermis by fibrocytes, forming a dense network of collagen-like fibers which are not resorbed but persist as a permanent scar;
- (3) encapsulation of foreign material too large to be phagocytized, forming melanized nodules in adjacent tissue;
- (4) involution of the epidermis into the wound and secretion of a cuticular layer similar to the normal external cuticle.

This entire process of wound repair seen in crustaceans is generally similar to that seen in insects—particularly rapid hemocytic and fibroblastic infiltration, encapsulations which result in formation of melanized nodules, and the migration of the epidermis into the wound, with subsequent cuticle development (Fontaine and Lightner 1975). The presence and activity of chitinoclastic microorganisms can confound the final stages of wound repair in crustaceans, however, and can lead to progressively severe manifestations of shell disease, either because of the extent of original damage or the failure of the animal to respond adequately in terms of cellular defenses (which include accelerated chitin synthesis and deposition).

#### Hypothesis IV.

*Pollutants (or other stressors) may foster the development and increase the severity of one form of shell disease resulting from disturbed metabolism of chitin.* Small focal lesions form at vulnerable sites (cuticular pores) and expand, as a consequence of activity of chitin-destroying microorganisms, to produce gross ulcer-like, penetrating lesions.<sup>1</sup> This form of shell disease seems distinct from lesions resulting from trauma (abrasion or mechanical disruption of the cuticle) with subsequent invasion of the exoskeleton by chitin-destroying microorganisms.

Bullis et al. (1988), reporting on shell disease in samples of red crabs, *Chaceon quinque-dens*, were the first to point out two forms of the disease in that species: (1) a random unilateral hyperpigmentation associated with apparent abrasions or other injuries, and (2) bilateral lesions which "... appeared to evolve as hyperpigmentation of microscopic sensory organelles on the surface of the carapace." The pigmented areas of type 2 "... became enlarged, confluent, and occasionally resulted in shell defects." Observations of shell disease in other crabs suggest that areas of melanization are characterized also by increased friability of the cuticle, leading to its rupture, fracture, or fragmentation, which can produce large areas in which the exoskeleton is simply absent (Fig. 1). Such a phenomenon has been seen or reported in lobsters (*Homarus americanus*), rock crabs (*Cancer irroratus*), and Jonah crabs (*Cancer borealis*) from the New York Bight apex (Pearce 1972, Sawyer, personal communication, 1989<sup>2</sup>) and from blue crabs (*Callinectes sapidus*) from Pamlico Sound (Engel and Noga 1989).

Shell disease—especially that characterized by extensive exo-



Figure 1. Blue crab, *Callinectes sapidus*, from the Pamlico River, North Carolina, showing extensive area of carapace loss (photograph courtesy of Dr. D. W. Engel).

skeletal damage—may, therefore, be part of a continuous process of degradation of the epicuticle by indigenous epiphytic lipolytic microorganisms, accompanied by chitin destruction in underlying cuticle by chitinoclastic bacteria (Baross et al. 1978). Large lesions may thus result from extensive bacterial invasion of the chitinous cuticular layers *without extensive initial epicuticular disruption*. This, of course, does not preclude the development of a background level of the disease resulting from mechanical damage to the cuticle by predators, or encounters with other members of the species—as was pointed out by Baross et al. (1978).

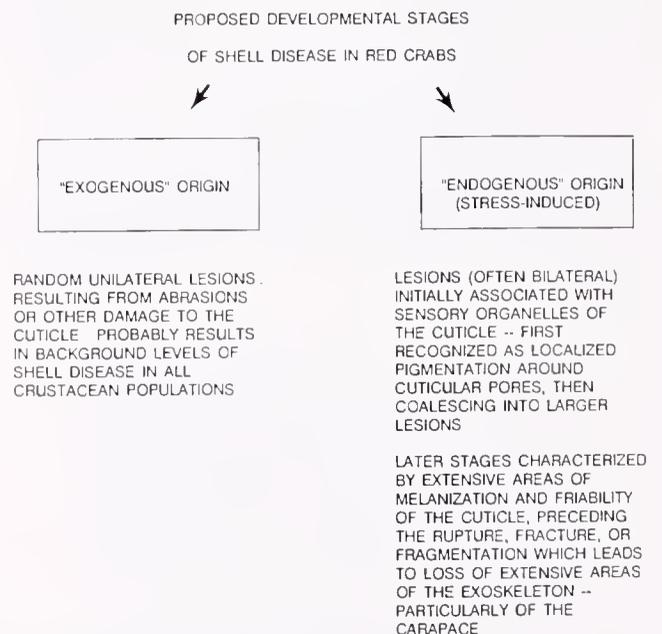


Figure 2. Dual pathways of shell disease (modified from Bullis et al., 1988).

<sup>1</sup>Note that "ulcers" are properly defined as "lesions of the skin and mucous surfaces that extend through the basement membrane," so use of this term in describing shell lesions in Crustacea is a slight misnomer. A better term would probably be "penetrating lesion."

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The essence of the dual pathway concept of Bullis et al. (1988) is illustrated in Figure 2. The "endogenous" form of the disease may be a consequence of immunosuppression (possibly because of stressors such as chemical pollutants) or of failure of metabolic processes associated with shell repair and maintenance.

#### Hypothesis V.

Shell disease may be less important in crustacean species with short-life spans (penaeid shrimp, blue crabs) than in longer-lived species (lobster, deepwater crabs), except in stressful environments (aquaculture, impoundments, shedding tanks, or chemically degraded habitats). Since shell disease appears to be progressive, its effects can be eliminated or minimized by molting—provided that secondary infections and destruction of underlying tissues have not developed, or that adhesions of old and new cuticles have not occurred. Animals which molt frequently or continuously throughout their life spans thus have repeated opportunities to cast off the effects of the disease (and the causative organisms as well).

#### Hypothesis VI.

Shell disease may occur in high prevalences in offshore deepwater crustaceans. Offshore populations are not as heavily fished, so they have high proportions of large, old individuals which molt infrequently or not at all—allowing the disease to progress to severe, readily observed lesions. Also, some deepwater crustaceans are long-lived, and shell disease is a normal concomitant of advancing age in crustaceans. Additionally, it is likely that different chitinoclastic microorganisms may dominate in deeper water. *Vibrios* tend to be the principal shell disease pathogens in shallow water, but, in one study of tanner crabs, the genus *Photobacterium*—a luminescent, deepwater bacterial group closely related to the *vibrios*—was the predominant isolate (Baross et al. 1978).

Evidence to support this hypothesis includes the following:

- Baross et al. (1978) found that 76% of adult female and 29% of adult male tanner crabs, *Chionoecetes tanneri*, taken in depths of 500–2000 m off the Oregon coast had shell disease lesions. (No lesions were seen in juveniles; females of this species do not molt after puberty.)
- Wenner et al. (1987) reported that 95% of golden crabs, *Chaceon femeri*, taken in depths of 300–700 m off the coasts of South Carolina and Georgia had blackened, abraded areas on the exoskeleton. Of these, 75% were intermolt and 19% pre-molt crabs.
- Young (1988) observed that 92% of red crabs, *Chaceon quinque-dens*, taken in three submarine canyons off the Middle Atlantic coast (Hudson, Block, Atlantic) in depths of 450–640 m had shell disease lesions—of which 21% were rated as mod-

erate to severe. (Severity of the condition was correlated directly with size.)

- According to newspaper accounts, lobster fishermen reported that up to 90% of red crabs from an offshore canyon (Block Canyon) of the Middle Atlantic Bight had shell disease in June 1988 (only 10% of lobsters were affected, according to the same reports.)

#### Hypothesis VII.

Shell disease is a controllable condition in captive or cultured crustacean populations. Effects of the disease in captive populations in cages or impoundments can be minimized by a series of management steps:

- do not retain impounded populations for a long time;
- remove dead individuals and cast exoskeletons frequently;
- remove diseased animals immediately;
- do not overstock the holding facility;
- provide adequate nutrition;
- handle the animals as little as possible, and do so carefully;
- provide shelter and hiding places;
- ensure adequate water flow, to provide oxygen, remove wastes, and prevent build-up of pathogenic bacteria;
- if feasible, filter and treat water with ultraviolet light.

Control of shell disease in aquaculture facilities should include all of the above steps, with emphasis on providing adequate nutrients, since Fisher et al. (1976, 1978) found a relationship of shell disease with poor nutrition. Additionally, prophylaxis and treatment of shell disease are feasible in aquaculture facilities. Dip and bath prophylaxis with malachite green, formalin, oxolinic acid, furanace, erythromycin, and streptomycin have been reported to be effective (Fisher et al. 1978, Tareen 1982, Brock 1983, El Gamal et al. 1986, Austin and Alderman 1987). Some of these drugs or chemicals may be useful also in treatment of infected animals, except when extensive shell perforations have developed, and underlying tissues have been destroyed. (It should be noted that some of these substances have not been approved for use with crustaceans being reared for human consumption.)

In summary, a substantial body of information exists about shell disease in marine crustaceans, although evidence supporting the hypotheses presented here may vary somewhat in "robustness." It seems clear that this is probably the most ubiquitous disease syndrome in marine crustaceans, and, as such, warrants attention. It is a disfiguring condition that can, in some instances, lead to mortalities—hence, it must be a factor of concern to those who manage commercial fisheries.

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## SHELL DISEASE IN THE ATLANTIC ROCK CRAB, *CANCER IRRORATUS* SAY, 1817, FROM THE NORTHEASTERN UNITED STATES

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**ABSTRACT** Gross observations were made on the prevalence of shell disease in the rock crab, *Cancer irroratus*, collected from coastal waters of the northeastern U.S. from 1973-1984. Discoloration or perforating lesions of the carapace and/or appendages ranged from less than 1% to 25% depending on the geographical location of collection sites, molting activity, and/or migratory habits. The prevalence of disease was higher in crabs collected near the New York 12-mile, and Philadelphia-Camden sewage-waste disposal sites, than in other sites ranging from Virginia to Maine. Direct observations on over 2,000 specimens suggest that a prevalence of shell disease in 5% or more of the *C. irroratus* population of the northeast region is an indication of seabottom degradation associated with sewage waste disposal practices.

**KEY WORDS:** rock crab, *Cancer irroratus*, shell disease

### INTRODUCTION

Possible effects of waste disposal practices in coastal waters of the northeastern United States have been studied using physical, chemical, and biological indicators. Changes in bottom sediments from clean sands or muds to black anoxic mucks have been documented at sewage disposal sites. Certain bacterial species have become resistant to antibiotics and heavy metals, and productive shellfish harvesting areas have been closed because of microbial contamination. More recently, commercial fishermen have complained of economic losses associated with the unsightly appearance of crabs and lobsters with shell lesions or black discoloration. The present report summarized gross observations on the prevalence of shell blackening and perforating lesions on the legs or carapace of *C. irroratus* captured in otter trawls during the period 1973-1984. Several thousand specimens were studied from collection sites near the New York 12-mile site and the Philadelphia-Camden offshore sewage waste disposal site. Small numbers were examined from sites away from disposal sites ranging from Maine to Virginia. Precise estimates of the prevalence of shell disease, or estimates of the effects of disease on mortalities were not possible since weakened or stressed crabs are subject to predation and rapid post-mortem decomposition. The study suggests, however, that shell disease in less than 5% of the population studied may be expected regardless of the role of seabottom contamination. The highest prevalence recorded in this study was 22% at the New York site, and 12% at the New Jersey "Mud Hole" station, both known to be seriously impacted by sewage disposal practices. Historical accounts of the significance of shell disease and its etiology in marine crustaceans, may be found in a recent review by Sindermann (1989).

### METHODS

Rock crabs taken near sewage disposal sites, from Sandy Hook Bay, New Jersey, and the "Mud Hole" were captured by making 20-30 min. trawls with a standard otter trawl. Crabs from other locations (Maine, Massachusetts, Maryland, Delaware, and Virginia) were provided by cooperating biologists or commercial fishermen who collected them in trawls or lobster pots. All specimens were measured to the nearest 0.5 cm., sexed, and observed for the presence of shell blackening and perforations of the carapace and appendages; those less than 6 cm. were not included in the study. Records were kept of collections that coincided with pre-, post-,

and intermolt activity. Data were analyzed to compare the prevalence of shell disease on a geographical and seasonal basis, presence or absence of molting, and as influenced by the ratio of males:females.

### RESULTS

*Prevalence of shell blackening or ulceration.* The prevalence of the two conditions was found to vary geographically, seasonally, and with molting activity. Migratory behavior associated with molting behavior was most pronounced in Sandy Hook Bay, New Jersey where crabs were absent in the late spring and summer months but abundant during cooler seasons. The Bay often yielded a male to female ratio of 100:1 during the winter-spring molt, and evidence of shell discoloration was noted only at the beginning of the molting period in late October or early November, or in small numbers of crabs that did not complete a successful molt by the end of the cycle in late April or early May. Accordingly, there were two peaks for the prevalence of disease in Bay crabs, 18% in May, and 13% in November (Table 1).

Migratory behavior was less pronounced at the deeper stations near the 12-mile disposal site and in the "Mud Hole," where crabs were present at all seasons of the year. Male:female ratios were variable throughout the year depending on migratory behavior, molting, and mating. Ratios ranged from approximately 2:1-14:1 while the cumulative yearly ratio was close to 4:1. Peak prevalence for shell disease at both locations was noted at two seasons as observed in Sandy Hook Bay. The 12-mile site had a peak of 22% in June and 21% in October, while the "Mud Hole" prevalence peaked at 10% in May and 12% in October (Table 1).

Rock crabs from the Philadelphia-Camden disposal site showed a prevalence of shell disease ranging from 3.5 to 6.5%. Collections were not made during the months of October-December, and only 10 specimens were examined during January-March. Studies at the site could be made only on occasions when overnight 2-3 day cruises were scheduled by other investigators. Data were not obtained on migratory activity in the population but molting and mating seasons appeared to occur in the summer months. In the July-September collection 139/582 (24%) of the specimens were in the soft- or post-molt condition. The newly molted group was made up of 60 males and 79 females, suggesting that this population was distinct from those that migrated into inland bays and molted during the fall and winter months.

Rock crabs captured inshore by commercial fishermen near

TABLE 1.

Prevalence of Shell Blackening and/or Ulceration in the Rock Crab, *Cancer irroratus*, from Waters of the Northeastern United States. (Cumulative data)

Location	No. Males	No. Females	Total Exam.	Shell Disease (%)
Sandy Hook Bay, NJ	506	103	609	54 (9)
12-mile Site, NY	369	103	472	64 (14)
"Mud Hole", NY/NJ	825	195	1020	79 (8)
Portland/Boothbay Harbor, ME	277	29	306	26 (8.5)
Phila.-Camden Site	548	488	1036	57 (5.5)
Inshore-DE, MD, VA	288	8	296	43 (14.5)

Note: Sandy Hook had peak values of 18.8% in May, and 13.4% in November.

12-mile site had peak of 21.8% in June and 21% in October.

"Mud Hole" had peaks of 10% in May and 12% in October.

Other Sites—insufficient data for analysis by season.

Delaware, Maryland, and Virginia during February–April had undergone a recent molt as evidenced by the soft or papershell condition of 57/94 specimens (60%); only 4 had shell discoloration (4%). Several other collections, from pots, were made near Ocean City, MD, during January–March and June–July. The January–March collection was made up of 103 late papershell males and none showed evidence of shell disease. The June–July collection was made up of 99 crabs. They were not sexed or examined for shell discoloration, but 39 of them had perforations of the shell or legs. Collections from Portland and Boothbay Harbor, Maine were made only in July. Shell perforation or discoloration was noted only on 26/306 (8.5%) of the specimens (Table 1).

*Perforations of the appendages.* Black perforations of the appendages that appeared to penetrate the shell were noted primarily on intermolt specimens. The prevalence of perforations on crabs from Sandy Hook Bay ranged up to 6%, those from the 12-mile site, up to 7%, and those from the "Mud Hole," up to 10% (Table 2). Perforations in crabs from the Philadelphia–Camden site ranged up to 9%. The number of crabs studied from Delaware, Maryland, and Virginia was too small to support an estimate of the prevalence of perforations from those areas. However, it was noted that among 56 crabs examined during February–March none had perforations, whereas 3/20 caught in April did have perforations. Among 103 crabs caught near Ocean City, MD, during January–March none had perforations, while 39/99 caught during June–July had either perforations or discoloration of the shell or appendages. Crabs captured in Maine, had perforations of the appendages in only 4/306 specimens examined (Table 2). All of the Maine crabs were in the intermolt condition; egg masses were noted on 16/29 females.

## DISCUSSION

Interest in shell blackening and perforating lesions in the rock crab, *Cancer irroratus*, is based primarily on a renewed concern about the possible effect of ocean pollution on commercially valu-

TABLE 2.

Prevalence of Lesions on Appendages of Rock Crab, *Cancer irroratus*, from Waters of Northeastern United States. (Cumulative Data)

Location	No. Males	No. Females	Total	Leg Lesions (%)
Sandy Hook Bay, NJ	506	103	609	22 (3.5) <sup>a</sup>
12-mile Site, NY	369	472	472	14 (3.0)
"Mud Hole" NY/NJ	825	195	1020	53 (5.0)
Portland/Boothbay Harbor, ME	277	29	306	4 (1.0)
Phila.-Camden site	548	488	1036	69 (6.5)
Inshore DE, MD, VA	228	8	296	Not recorded

Note: Sandy Hook had peak values of 6% in May and 5% in Dec.

12-mile Site had peak value of 7% in Oct.

"Mud Hole" had peak values of 10% in June, and 8–9% in Oct.–Dec.

Sandy hook—maximum shell and leg disease—24% in May.

12-mile Site—maximum prevalence—25% in June, and 29% in Oct.

"Mud Hole"—maximum prevalence of 19 and 18% in May and June, and 20% in Oct. and Dec.

able shellfish. Waters of the New York Bight are of special interest since previous and ongoing studies of bacteria, viruses, petroleum hydrocarbons, heavy metals, etc., have documented serious environmental degradation, especially in the Bight apex. Studies presented here are based on limited field observations made during the course of research on gill blackening as a consequence of bottom sediment contamination (Greig *et al.* 1982, Sawyer *et al.* 1985). Shell condition, as defined in our studies, was based on black, non-perforating blotches on the dorsal carapace, or the presence of focal perforations in the carapace or jointed appendages. There remains a need, however, to develop more specific indices for the size and number of blackened zones, and the association of perforating lesions with crab size. Although not specifically addressed in our study, it was noted that the majority of perforations were present on large 10–13 cm. crabs that probably had undergone a terminal molt. Similarly, it was not possible to determine whether perforations were due to chitinoclastic microorganisms, or due to old injuries. Although the numbers of crabs sampled away from known sewage-waste disposal sites were small, the data suggest that both conditions occur naturally in the marine environment at a background level of approximately 5%. Areas that are impacted by contaminants, on the other hand, may be expected to show prevalences of up to approximately 20%.

A survey of existing literature, combined with our observations, show that migratory behavior, and differences in mating periods between males and females, have a significant influence on shell discoloration and perforations. Crabs that have not reached the adult stage may molt several times during the same season, and adult males and females may molt at approximately the same, or at different months of the year. Our data show a distinct difference in molting periods for the nearshore population, and an overlap among males and females in deeper offshore waters. Reilly and Sails (1978) collected rock crabs from Block Island Sound to Nantucket Shoals. They reported that females over 60 mm. molted during November and December, males over 90

mm. from January to March, females between 50–70 mm. molted in June and July, and males between 40–60 mm. from May to July. Thus, when size is not taken into account, newly molted rock crabs may be found in New England waters throughout the year. Although we did not examine molting activity or shell disease in crabs less than 60 mm, we did note that inshore males and females over 60 mm, molted at the same seasons noted by Reilly and Saila (1978). In contrast to the inshore populations, we found that both intermolt crabs and newly molted adult male and female crabs were present at the same time in some of the deepwater collections. Comparative data suggest that deepwater populations may not undergo the extensive shoreward migrations noted with the inshore population.

Shell discoloration and/or perforating lesions in rock crab populations away from established ocean disposal sites needs to be studied more thoroughly. Our studies were limited to only a few collections and all seasons of the year were not represented. Preliminary data suggest, however, that perforation of the carapace and/or appendages is more common than shell discoloration or blackening in inshore *C. irroratus* in Maine waters. Ongoing research in other laboratories may show that such lesions may sometimes be caused by bacteria that may or may not be associated with sewage wastes. Crabs collected inshore near Delaware, Maryland, and Virginia were not representative of all seasons of the year. Our data did show, however, that crabs migrated shoreward and molted during the winter seasons as did those in New York and New Jersey. Sediment contamination by sewage wastes from coastal outfalls from each of the 3 states is well known, and further studies on fish and shellfish in these areas should be carried out. Haeffner (1976) made extensive studies on the distribution and molting of rock crabs in the mid-Atlantic Bight. He noted that *C.*

*irroratus* made extensive migrations into the waters of the Chesapeake Bay to molt during the winter months. Therefore, it appears that seasonal influences on inshore and offshore populations may account for differences in the prevalence and geographical distribution of shell disease. Specifically, shoreward migrations of nearshore populations in late fall and winter, and associated molting, would cause a decrease in the estimated prevalence of shell disease during those seasons. Similarly, simultaneous molting of males and females in offshore waters would require more extensive studies to determine the seasons in which most of the population is in the intermolt condition. Shell disease may be a useful indicator for one of the impacts of sewage pollution in coastal and offshore waters when over 5% of the population is affected. However, it remains to be determined whether high prevalences are associated with microbial activity, toxic chemicals, or other environmental contaminants. Specific references to the present state of knowledge concerning shell disease are given in the review by Sindermann (1989).

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## PREVALENCE AND SEVERITY OF SHELL DISEASE AMONG DEEP-SEA RED CRABS (*CHACEON QUINQUEDENS*, SMITH, 1879) IN RELATION TO OCEAN DUMPING OF SEWAGE SLUDGE

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**ABSTRACT** The extent and severity of shell disease among Middle Atlantic Bight deep-sea red crabs (*Chaceon quinquedens*) from six offshore sites of varying distance from the 106-Mile Sewage Sludge Dumpsite were assessed by evaluating each individual according to predetermined rating criteria. Additional specimens dating to the late nineteenth century, deposited in the Smithsonian Institution crustacean collection, were also examined and rated in the same manner.

Overall disease prevalences among collected crab samples ranged from 86% to 100%, and Smithsonian Institution samples varied from 69% to 100%, with 0% to 60% in the moderately to severely diseased range. Appearance of the shell disease ranged from very small black spots to large grey to black patches covering a substantial portion of the carapace, often arranged in a bilaterally symmetrical pattern. Disease severity increased with size of individuals.

**KEY WORDS:** *Chaceon*, shell disease, ocean dumping, sewage, red crab

### INTRODUCTION

Shellfishermen working the waters off the continental shelf near the New York Bight have asserted that an unreasonably high percentage of their crab and lobster catches have been affected by shell disease, which they attribute to the disposal of municipal sewage sludge at the 106-Mile Sewage Sludge Dumpsite (Fig. 1). The New York Bight is the area extending from Cape May, New Jersey, to Montauk Point, Long Island, and seaward about 190 km to the continental shelf break.

The red crab, *Chaceon quinquedens* (formerly *Geryon quinquedens*) (Manning and Holthuis 1989), was chosen as the subject of this study because: 1) it was one of the species that some commercial fishermen alleged was adversely affected by the dumping of sewage sludge at the 106-Mile site; 2) red crabs inhabit deep waters, approaching the depths at the 106-Mile site, thus the range of the red crab is in the proximity of the dumpsite; and 3) the narrow range of the red crab precludes the possibility that crabs migrate long distances inshore. Also, there is no evidence that red crabs migrate substantially along depth contours, so red crabs are not subject to the contaminant loads of many areas as are migrating species (Haefner 1978).

Shell disease is the degradation of crustacean integument by a variety of microorganisms which invade the chitinous layers of the exoskeleton (Getchell 1989). It is characterized by "a progressive chitinolysis and necrosis of the exoskeleton of aquatic crustaceans" (Rosen 1970), and in general, is observable as darkened lesions on the exterior of the shell. Although no single organism has been universally regarded as the causative agent, investigators have isolated a variety of chitinoclastic bacteria and fungi in shell lesions of numerous crustacean species (Cook and Lofton 1973, Fisher et al. 1978, Murchelano 1982), and, in fact, the condition is generally regarded as a "disease syndrome" rather than as a discrete disease (Sindermann 1989).

The 106-Mile site has been an important and increasingly utilized element of the New York-New Jersey metropolitan waste disposal system. Beginning in 1986, the dumping of sewage

sludge was shifted incrementally from an inshore dumpsite to the 106-Mile dumpsite, with the transition being completed in December of 1987 (USEPA 1989, NOAA 1988). The site, located just off the continental shelf break, has a total area of approximately 100 square nautical miles and ranges in depth from 1550 m to 2750 m (Simpson et al. 1981). The mixed layer extends to 20 m during the summer and 100 m in winter (O'Connor et al. 1985). Until the 1990 Congressional mandate to cease ocean dumping, nine sewerage authorities in New Jersey and New York dumped an estimated  $7.5 \times 10^6 \text{ m}^3$  at the site annually (Pkehrberger 1989).

This investigation was undertaken to quantify the prevalence and severity of shell disease among deep-sea red crabs inhabiting the region surrounding the 106-Mile site, and to begin a data base that can be used in further study of this phenomenon in relation to ocean waste disposal. It should be noted that this was intended only as a preliminary study and was limited in some cases by the unavailability of adequate sample sizes.

### METHODS

Visual examinations were made of red crab samples taken from various locations over time. A disease severity rating system was devised so that comparisons among the samples could be made, thus facilitating observations of areal or temporal trends relating to disease prevalence and severity. Results of chemical analyses of hepatopancreas tissue of crab samples from three locations in the New York Bight have been reported elsewhere (Young 1990).

Initial samples were collected aboard the National Oceanic and Atmospheric Administration (NOAA) ship ALBATROSS IV on 29 and 30 June, 1988, from the vicinities of three submarine canyons (Fig. 1) along the continental shelf edge near the New York Bight. Thirty-minute bottom trawls were made at 3.7 to 4.6 km/hr (2 to 2.5 knots) using a 3/4-size try-net apparatus. As many specimens as possible were examined immediately upon capture for the presence of shell disease. Each animal was sketched to indicate the affected areas of the body, and a disease severity rating was assigned to each individual using the criteria listed in Table 1, which were developed after an initial examination of about 100 crabs. The severity ratings range from one to five, with a "1" indicating that no shell disease was visible to the naked eye, and a "5" signifying a severely diseased individual. A zero rating

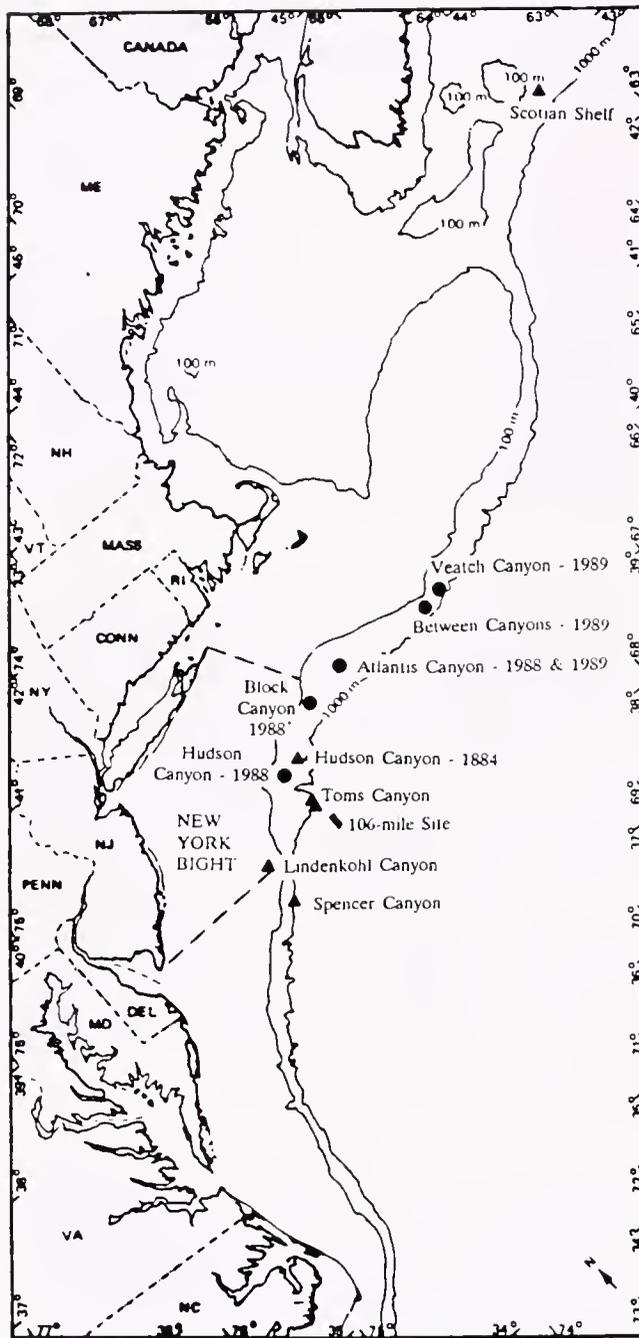


Figure 1. Location map: ● = Albatross IV and TAURUS samples; ▲ = Smithsonian Institution samples.

was not used to describe individuals lacking apparent signs of shell disease because the initial stages of shell disease are visible only through a microscope, and a zero rating might give the erroneous impression that the animal was absolutely free of any shell disease when in fact it may not have been. Carapace width, sex, and shell texture were also recorded. All the crabs that could not be inspected promptly were placed in plastic bags and frozen aboard ship for later examination. The total numbers (subsets of the total catches) of crabs examined from Hudson, Block, and Atlantis Canyons were 202, 77, and 110 respectively.

An additional set of samples was collected and inspected aboard the F/V TAURUS in September, 1989. These crabs were collected from the waters of Atlantis and Veatch Canyons, as shown in Figure 1. The TAURUS, a fishing vessel sailing out of Fall River, Massachusetts, is dedicated to deep-sea red crab fishing. Lines of 150 wooden traps, measuring 119 × 81 × 56 cm (47 × 32 × 22 in.) were laid out across the ocean floor. These traps are typically retrieved between 19 and 24 hours later (G. Rivinius, pers. comm.). Extra time needed to recover lost gear, however, increased the time that some traplines remained on the bottom.

In order to evaluate temporal trends in shell disease prevalence among red crabs, specimens from the crustacean collection at the Smithsonian Institution in Washington, D.C. were examined. Forty-eight specimens collected near the Hudson Canyon during the summer of 1884 aboard the ALBATROSS I were examined. Seven additional specimens collected from Toms Canyon to Spencer Canyon aboard the same vessel in the autumn of that year were also inspected. Two more recent small samples were also included in the study. The first of these was a sample of 13 collected in 1976 and 1977 near the Linden Kohl Canyon. The other sample consisted of five individuals caught south of Nova Scotia, Canada, in 1981 and 1985. It is important to note that because there is no record of how any of the Smithsonian samples were selected for preservation, it is not known whether the presence or absence of shell disease influenced the decision to save each individual crab.

## RESULTS

Although shell disease was present in each of the samples examined, most of the individuals were only slightly affected, as defined in the disease severity rating criteria given in Table 1. Comparatively few were moderately affected and fewer still showed signs of advanced disease. Disease prevalences are presented in Table 2.

At the 1988 Hudson Canyon site, 92% (185/202) of the red crabs examined exhibited at least some degree of shell disease. Seventy-eight percent were very slightly or slightly affected, 8% moderately affected, and 5% severely diseased. Of the individuals assigned a disease severity rating of "4" or "5," 85% had a

TABLE 1.

Criteria for disease severity ratings.

Rating Number	Disease Severity	Criteria
1	Imperceptible	Absence of visible spots
2	Very slight	<10 small spots or only small light grey patches
3	Slight	>10 small spots, but <10% of body blackened
4	Moderate	Large areas affected with blackening over 10-50% of body
5	Severe	Blackening over >50% of body; or open lesions present; or old, blackened amputation sites

TABLE 2.  
Prevalence and severity of shell disease among deep-sea red crabs, *Chaceon quinquegens*.

Severity Rating	Hudson 1988	Block 1988	Atlantis 1988	Atlantis 1989	Between 1989	Veatch 1989	Hudson 1884	Toms-Spencer 1884	Lindenkohl 1976-77	Scotia 1981 & 1985
1	17	6	10	0	1	0	9	0	4	0
2	77	15	31	7	11	5	24	0	4	0
3	81	33	48	23	22	28	15	4	3	2
4	16	13	16	11	7	10	0	1	2	3
5	11	10	5	3	1	3	0	2	0	0
Totals	202	77	110	44	42	46	48	7	13	5

carapace width of 9.0 cm or greater. The prevalence at the 1988 Block Canyon location was 92% (71/77), with 62% rated a "2" or "3," 17% assigned a "4" rating, and 13% given a "5" rating. All of the crabs rated moderately or severely affected measured 9.0 cm wide or more. Ninety-one percent (100/110) of the 1988 Atlantis Canyon specimens showed signs of shell disease. Seventy-two percent of the individuals received a "2" or "3" rating, 15% a "4," 5% a "5" rating, and 86% that were moderately or severely affected were 9.0 cm or larger. All individuals among the 1989 Atlantis Canyon sample showed at least some signs of disease, with 68% of the individuals very slightly or slightly affected, 25% moderately affected, and 7% severely diseased. Ninety-eight percent (41/42) of the specimens caught between Atlantis and Veatch Canyons exhibited shell disease. Individuals assigned a "2" or "3" rating comprised 79% of the total; those rated "4" and "5" made up 17% and 2% of the sample respectively. Among the Veatch Canyon sample, 72% were rated a "2" or "3," 22% were assigned a "4" rating, and 7% a "5" rating. Because the TAURUS fished deeper waters than the ALBATROSS IV, in order to harvest predominantly larger males, only three individuals among the 132 examined aboard the TAURUS fell below 9.0 cm, making size comparisons among these samples difficult.

The remaining samples examined were those housed in the Smithsonian Institution collection. Although eighty-one (39/48) percent of the 1884 Hudson Canyon specimens showed at least some signs of shell disease, none of the crabs was assigned a severity rating greater than "3." In general, disease severity increased with animal size. Ninety-three percent of the individuals assigned a "3" rating were 9.0 cm or larger. The Toms-Spencer sample had a 100% disease prevalence (7/7), with 57% slightly affected and 43% moderately to severely affected. All of the individuals in this sample had a carapace width of 9.0 cm or greater. Sixty-nine percent (9/13) of the Lindenkohl Canyon specimens exhibited shell disease. Of these, 54% fell into either the very slightly or the slightly affected categories. The other 15% were moderately affected. All of the specimens rated moderately affected were larger than 9.0 cm. At the Scotian Shelf site, 100% (5/5) of the crabs examined were slightly to moderately diseased.

## DISCUSSION

### Symmetry of Shell Disease

Very often, signs of shell erosion were found to be bilaterally symmetrical. This can sometimes be explained as regions receiving equal amounts of abrasion, thus equally wearing away the epicuticle and allowing infection by chitinoelastic organisms. For

example, necrosis of the shell tissue was sometimes observed where the ventral side of the coxae rub along the sea floor. More commonly, body parts of the animal itself which rub together were affected. For instance, where the merus of the first walking leg comes into contact with the cheliped, or where the merus of the cheliped rubs against the sub-hepatic region of the carapace, grey or black areas were often found. The effect of abrasion of the epicuticle may be exacerbated by the fact that crustaceans may not have the ability to repair damaged epicuticle (Halcrow 1988). Other examples of bilateral symmetry cannot be explained by abrasion of the epicuticle. Areas of the branchial or hepatic regions, or the points along the edge of the carapace, are often symmetrically blackened, but are not continually in contact with

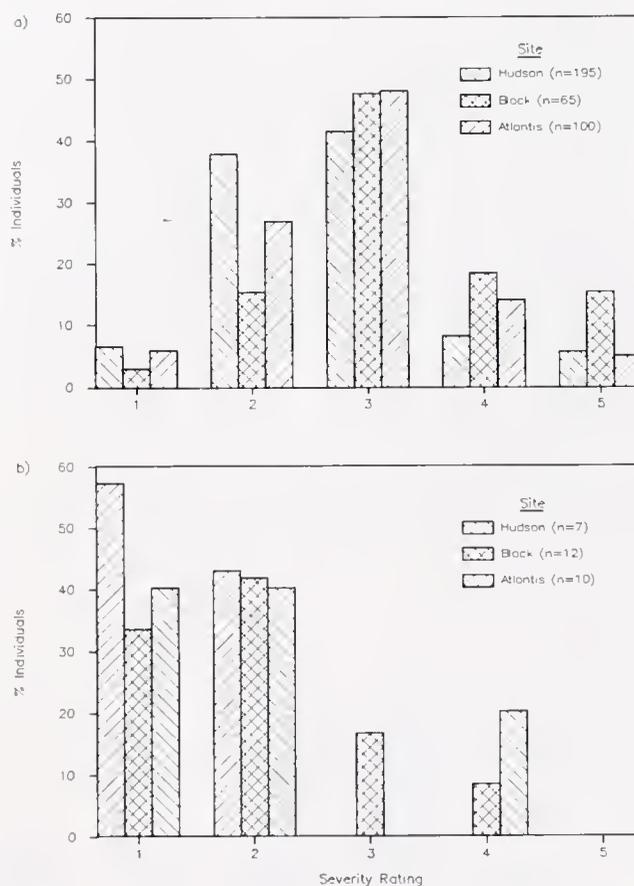


Figure 2. a) Disease prevalence and severity among hard-shelled crabs; b) Disease prevalence and severity among soft-shelled crabs.

the sea floor or other body parts. This suggests a second means of infection such as the entry of chitinolytic microorganisms through hypodermal ducts or setal pores (Fisher et al. 1978).

Shell erosion was not, however, always bilaterally symmetrical. The infection of some non-symmetric areas of the exoskeleton can easily be explained by abrasion or partial crushing of the shell, thus allowing access to the inner layers by chitinoclastic organisms. Other regions that had been affected non-symmetrically had either become infected before their bilateral counterpart or the epicuticular defense was breached by another route. Baross and Tester (1975, cited in Fisher et al. 1978) suggest that chitinoclastic microorganisms may gain access to the chitinous layers with the aid of lipolytic bacteria.

#### Molting and Size Considerations

Crustaceans suffering from shell disease may overcome the condition by molting, thus forming a new, uninfected shell (McLeese 1965, Rosen 1970). A comparison between hard-shelled and soft-shelled ALBATROSS IV specimens (Fig. 2) clearly demonstrates the red crab's ability to rid itself of a diseased exoskeleton and start fresh with a new one. Nearly all of the soft-shelled individuals examined were either free of signs of shell disease or only slightly affected, with few small spots visible. The few exceptions were almost all individuals which had suffered physical damage to the new shell, such as a crushed or torn area, which allowed the entry of disease-causing agents into the chitinous layers of the shell. This is not surprising, as newly molted individuals are vulnerable to damage without the protection of a hardened shell.

Shell disease severity in red crabs seems to be associated at least in part with animal size (Fig. 3). Of the modern samples, the Hudson Canyon population was comprised of the smallest individuals on average, and also had the lowest mean severity rating (Young 1990). Block Canyon, on the other hand, had the highest percentage of larger individuals and also the highest prevalence of "4" and "5" ratings. This relationship is possibly related to molting, since the frequency of molting may decrease among larger individuals (Warner 1977) and longer intermolt periods allow more time for the chitinoclastic microorganisms to spread throughout the shell of their host. Tagging studies by Lux et al. (1982) showed that the molting frequency of adult red crabs is quite low, sometimes as long as seven years.

#### Oceanographic Conditions

Modelling and survey studies have indicated that, in general, sludge dumped at the 106-Mile site disperses along the continental

shelf in a southwesterly direction. A model developed by Paul et al. (1989) predicted dilutions of  $10^6$  in surface waters within 10 km of the dumpsite, and analyses of current data indicate that wastes should be transported away from the site at a mean rate of at least  $10 \text{ km} \cdot \text{d}^{-1}$ . Vertical transport of sewage sludge is affected by several factors, including particle size and density of the sludge, incorporation into biota, and the presence of gradient surfaces in the water column. Because sewage sludge is near neutral density (O'Connor et al. 1985), a major portion of it may stay in the upper mixed layer where planktonic organisms can assimilate it. The existence of a pycnocline has been shown to impede the descent of particles through the water column. Proni and Hansen (1981) demonstrated that particles accumulate along density gradients. A secondary role of the pycnocline is that it affects the dilution rate of sewage sludge. After the breakdown of the seasonal pycnocline in winter, dilution may increase by an order of magnitude (Swanson et al. 1985). Thus, benthic organisms, including the red crab, may be exposed to negligible quantities of sewage sludge.

#### CONCLUSIONS

Comparisons using a G-test of independence revealed several differences in disease severity among crab samples. Severity of the disease syndrome fluctuates over time. In two cases, a significant difference in disease severity was detected between samples collected from very nearly the same site. In the first instance, the sample caught near the Hudson Canyon in 1884 was found to be significantly less diseased ( $0.001 < P < 0.01$ ) than the sample caught in the same waters in 1988. The second case, in which the 1989 Atlantis Canyon sample differed significantly ( $0.01 < P < 0.05$ ) from the 1988 sample, indicates that oscillations in disease severity due to natural variation may occur over shorter time spans as well, although much more sampling would be required to affirm this supposition.

Shell disease severity may vary with location. A significant difference in disease severity was apparent among the 1988 ALBATROSS IV samples, with the Block Canyon sample evidently more diseased than the others. However, there was no significant variation among the 1989 TAURUS samples ( $P < 0.05$ ).

There appears to be an association between disease severity and animal size, with disease severity increasing among larger individuals. This supports the supposition that red crabs may molt less frequently or not at all upon reaching maturity. In fact, it may be that the Block Canyon sample appeared more diseased than the other two 1988 samples because the average size of the crabs from this sample was considerably larger.

Pauley (1975) stated that "for infectious disease to occur, a potential pathogen must exist, a suitable host must be present, and the proper environmental conditions must pervade that will cause either an increased virulence of the pathogen or an increased susceptibility of the host." As it is given that the pathogens and hosts are present, it is the last of these conditions that still requires elucidation in relation to shell disease prevalence among the Middle Atlantic Bight red crab population. Despite the voids in our understanding of the disease, however, the prevailing presence of severe shell disease among individuals captured from other areas and at other times suggests that the dumping of sewage sludge may not substantially foster the condition. Shell disease occurs among red crab populations of the Middle Atlantic Bight, and has done so long before the commencement of sewage sludge dumping in the Bight, and, in fact, before the initiation of any anthropogenic

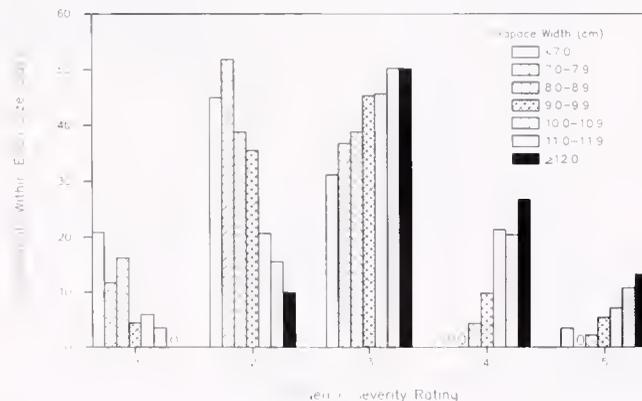


Figure 3. Size-percent frequency distribution for all samples.

impacts which may have been brought on by the Industrial Revolution. While the 1884 autumn sample collected from Toms Canyon to Spencer Canyon is unfortunately small, it nevertheless indicates the presence of shell disease near the vicinity of the present deep-water dumpsite years before the dumping of sewage sludge was initiated. The fact that these crabs were collected only a few months after the 1884 Hudson Canyon specimens suggests that either they were part of a separate population or that shell disease among red crabs may spread rapidly under certain conditions. Furthermore, the more recent specimens caught south of Nova Scotia indicate that shell disease occurs among red crabs inhabiting distant waters as well.

This work was designed as a preliminary study of shell disease in relation to ocean dumping of sewage sludge. In order to better understand the causes of shell disease, additional studies which employ larger sample sizes and consider seasonal variations are

needed. However, the evidence presented here brings to question whether, in fact, shell disease is indeed escalating among red crabs and suggests that sewage sludge dumping at the 106-Mile site is not the primary cause of shell disease in red crabs if in fact it contributes to the disease incidence at all.

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## SHELL DISEASE IN MARINE CRUSTACEANS: CONCLUDING REMARKS

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Two major conclusions can be drawn from the papers presented in this special session. First, shell disease is a common phenomenon in both wild and captive crustacean populations of western Atlantic ecosystems. Second, this condition is associated with multiple environmental scenarios. Evidence has been presented both in this meeting and in other published reports that shell disease may be an indicator of stressful environments. However, to control shell disease in captive populations and to use it as a meaningful indicator in natural environments, we must understand its pathogenesis beyond the vague notion that it is due to "stress."

Thus, there needs to be a more thorough understanding of shell-protecting mechanisms. Specifically, how does a primarily acellular biological structure exposed to a high concentration of potentially pathogenic/degradative agents maintain its integrity? Of course, this question has relevance not only to shell disease but to how the so-called "fouling organisms," including various bacteria, fungi, algae and invertebrates, can colonize these same surfaces. Many of these organisms have also been linked to poor water quality.

### MECHANISMS OF INVASION AND DEFENSE

The crustacean shell (cuticle) consists of an outer lipid-containing epicuticle, a middle, calcified, chitinous procuticle and an inner epidermis (Stevenson 1985). Intact immune cells do not inhabit the epicuticle or procuticle, suggesting that humoral immunity may be very important in shell defenses. Transport of such factors might occur via epidermal cell cytoplasm, which ramifies through pore canals (Stevenson 1985). Tegumental gland ducts, which also extend to the cuticle's surface, contain antibacterial agglutinins (Stagner and Redmond 1975) and phenol oxidase (Stevenson and Schneider 1962) in some arthropods.

#### *Causes of Shell Damage*

##### **Mechanical Trauma**

Trauma, as may occur in overcrowded conditions, has been suggested as a risk factor in shell disease development (Johnson 1983, Sindermann 1989). While physical damage may be important in facilitating lesion initiation, it does not appear to be solely capable of inducing progressive shell disease lesions in healthy animals (Cipriani et al. 1980, Noga et al. 1990).

##### **Microbes**

High environmental pathogen load that presumably causes increased risk of infection has been hypothesized as a contributor to shell disease development (Sindermann 1989). But, demonstration of a shell "infection" requires that one identify pathogens that are unique to shell disease lesions or are at least present in greater numbers in diseased animals. While some investigators have inferred that shell disease lesions have more bacteria than the normal carapace (Anderson and Conroy 1968, Cipriani et al. 1980), there

is little quantitative data to support these claims. The normal carapace is heavily colonized by microbes (Baross et al. 1978, Noga et al. 1990) and there has been little quantitative examination of the relative numbers of putative pathogens present in shell disease lesions compared to normal carapace. Lipolytic, proteolytic or chitinolytic bacteria have been isolated from shell disease lesions, but these enzymes are also common properties of environmental isolates (Hood and Meyers 1974, Lear 1963) and the normal shell microflora (Cipriani et al. 1980).

##### **Chemical Modification of the Shell**

Do certain environmental conditions facilitate shell degradation? The water bathing the shell could be considered an incubation medium that may stimulate or inhibit microbes in various ways, causing changes in microflora composition or metabolic activity that may affect shell integrity. Most crustaceans affected by shell disease are benthic species and direct contact with various sediment contaminants may also be important.

##### *Host Protection Mechanisms*

##### **Physical**

Physical defenses in the crustacean cuticle include a lipid-containing outer coat and an inner layer of chitin with its mineral matrix. However, this alone is obviously not sufficient to defend against shell disease (See Healing Responses).

##### **Immunological**

Hemocytes or other cells do not appear to play a large role in crustacean shell defense unless erosion extends through the entire carapace, but chemicals released by such cells and transported into the shell may be important. There has been very little examination for chemical agents that may defend against shell-invading organisms. The prophenoloxidase cascade is an important defense for freshwater crayfish against fungal invasion of the shell (Soderhall and Smith 1986). A hemolymph factor in blue crabs is active against many of the bacteria colonizing crab shell (Noga et al. 1990).

##### **Biochemical**

Several classes of compounds might defend against pathogens. Anti-nutrients, which bind essential elements such as iron or vitamins, could prevent shell invasion by inhibiting pathogen growth into the shell. Enzyme inhibitors for chitinases, lipases and proteases would not necessarily kill or otherwise harm the resident flora, but simply prevent them from using degradative chemicals on the shell. Hemolymph of crayfish that are resistant to invasive fungi inhibits chitinase activity of that pathogen (Unestam and Weiss 1970). Anti-colonization factors may prevent the attachment of pathogens by producing chemicals that repel attachment or by promoting colonization of a "normal" flora which excludes

pathogens. Gil-Turnes et al. (1989) showed that a symbiotic bacterium that is normally part of the endogenous shell flora protected shrimp larvae by producing an antibiotic that inhibited a pathogenic fungus.

### Healing Responses

Healing and repair, in response to normal day-to-day trauma, may be inhibited, resulting in defective chitin, lipid and/or protein synthesis, or failure of proper mineralization. Difluorobenzuron, a chitin inhibitor that is used as an insecticide, inhibits carapace formation, resulting in a softening of the shell and resultant development of shell lesions (Weiss et al. 1987).

### FUTURE RESEARCH NEEDS

We know very little about shell disease, so the list of future research needs could be quite long. From a basic biological standpoint, the relative unimportance of cellular defenses in the crustacean carapace make it an attractive model for understanding how chemicals modulate host protection. From a more practical standpoint, I think that several questions need to be addressed, including:

1) What common modes of pathogenesis (if any) link the various risk factors associated with shell disease?

Many types of environmental perturbations have been associated with shell disease. Identifying common mechanisms that lead to these final clinical manifestations would greatly enhance the value of shell disease as an indicator of environmental stress.

2) What does shell disease tell us (if anything) about the overall health of the affected shellfish populations?

There is little information on the ecological impact of shell

disease on any wild crustacean population (Sindermann 1989), although it is known to cause mortality in cultured organisms (Fisher et al. 197\*). Does shell disease make the host an easier target for predators? Does it cause slower growth rates or other sublethal effects? Does it increase risk of more lethal infections such as bacteremia? Exoskeletal lesions and bacteremia are associated with similar pathogens (Krantz et al. 1969, Brinkley et al. 1976).

3) What does shell disease tell us (if anything) about the overall health of that aquatic ecosystem (indicator value)?

Again, most of the data at present is inferential, with little direct linkage to any specific pollutants. Shell disease is readily visible in affected animals, making it fairly easy to quantitate and an attractive candidate for monitoring programs. Also, the implicit importance of chemicals in defense also suggests that chemical markers of this defense may also be useful indicators of stress.

In order to answer these questions, a multidisciplinary approach to studying shell disease must be taken that includes, first, developing a standard, reproducible model of shell disease that can be easily used by multiple investigators to study the syndrome; second, designing an integrated plan to quantify the effects of ecologically relevant stressors on the development of shell disease; third, determining the physiological, biochemical and microbiological changes that lead to shell disease under these ecological conditions and finally, applying the model to field situations.

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**ABSTRACTS OF TECHNICAL PAPERS**

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**HEMOLYMPH CHANGES IN DUNGENESS CRAB CAUSED BY INFECTION WITH THE CILIATED PROTOZOAN PARASITE, *MESANOPHRYX* SP.** Therese M. Armetta\*, Washington Department of Fisheries, Point Whitney Shellfish Laboratory, Brinnon, WA 98320.

Hemolymph from healthy Dungeness crabs, *Cancer magister*, was compared with that from crabs artificially infected with the ciliate, *Mesanophryx* sp. All crabs sampled were mature, intermolt males. Total and differential hemocyte counts, as well as total serum protein, glucose, and hemocyanin concentrations were measured.

Hemocytes of parasitized crabs were eliminated by the ciliates by the tenth day of the disease; however, eosinophilic granulocytes displayed an increase in relative numbers through the sixth day of the disease. Total protein and hemocyanin concentrations of infected crabs were lower than those of controls; however, glucose concentrations were higher. Analysis of serum by polyacrylamide gel electrophoresis showed infected crabs had altered patterns of total protein, including several bands that were not present in healthy animals.

**MANAGING PACIFIC RAZOR CLAM, *SILIQUA PATULA*, STOCKS AFFECTED BY NUCLEAR INCLUSION X (NIX).** Dan L. Ayres,\* and Donald D. Simons, Washington State Department of Fisheries, 331 State Highway 12, Montesano, WA. 98563.

The Pacific razor clam, *Siliqua patula*, has long been one of the most sought after shellfish resources in Washington State. Beginning in the late 1800's as a commercial fishery, razor clam digging later evolved into a popular recreational fishery. By the late 1970's and early 1980's clam diggers were making up to nearly one million user trips a year.

In 1983 during routine population sampling, Washington Department of Fisheries (WDF) biologists discovered that nearly 95% of the Washington razor clam population had inexplicably disappeared. Scientists at Battelle Northwest Marine Laboratories found that a unidentified gill parasite, later labeled Nuclear Inclusion X (NIX), was the most likely cause. The population quickly recovered to a level that allowed some sport harvest beginning in 1985, although seasons are now measured in days rather than months.

Since the initial discovery, WDF in concert with Battelle, has monitored the infection levels of NIX in razor clams on a monthly basis. NIX continues to be present in 100% of the Washington razor clams tested.

With the help of local state legislators, WDF secured a supplemental State budget appropriation to begin a cooperative study by WDF, Battelle and the University of Washington.

This paper will outline the importance of the razor clam fishery to the State of Washington, the NIX problem faced by WDF resource managers and the work being accomplished by the NIX Investigations Team.

**IMPROVING *PANOPEA ABRUPTA* SURVIVAL BY PLANTING IN TUBES.** J. Hal Beattie and B. Blake, Washington Department of Fisheries, 1000 Point Whitney Road, Brinnon, WA 98320; J. P. Davis, University of Washington, School of Fisheries and Ocean Science, Seattle, WA 98195.

This work explores methods by which geoduck seed can be successfully planted in the lower intertidal area. Laboratory work by Leitman et al. has demonstrated the decimating effects predators can have on newly planted geoduck juveniles; predation by crabs (*Cancer gracilis*) removed at least 30% of the geoducks within 48 hours. Similarly, in surveying subtidal areas two years after planting, Washington Department of Fisheries divers have recorded survival rates of less than one percent. On the other hand, work by Shuman demonstrated 70% survival of geoduck juveniles grown intertidally when protected by netting and plastic containers.

During 1990, in order to test an alternate planting method, we put geoduck juveniles averaging 6 mm shell length inside of twelve inch diameter PVC pipe, twelve inches long. The tubes were buried in the substrate with only one inch exposed. The top of the tube was covered with netting. Survival over the first ten and one half months averaged thirty percent at a site on Bainbridge Island and twenty percent at a site near the Hood Canal Bridge. In 1991, we modified and expanded this work by planting 800 tubes on four State Park beaches.

**DISCRIMINATING KUMAMOTO AND PACIFIC OYSTERS USING MOLECULAR MARKERS.** Michael A. Banks,\* Daniel J. McGoldrick, and Dennis Hedgecock, Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923.

The Kumamoto oyster, widely regarded as a race or variety of the Pacific oyster *Crassostrea gigas*, deserves status as a separate species, *C. sikamea* Ahmed 1975, on the basis of (1) the inability of Kumamoto oyster sperm to fertilize Pacific oyster eggs; (2) numerous fixed differences in allozymes between the two oysters and an average genetic distance of 0.4; and (3) a 2% difference in the nucleotide sequence of the mitochondrial (mt) DNA coding for the large ribosomal RNA (16S) subunit. Molecular genetic differences suggest that the two species diverged from each other at least 1 million years ago. Morphological and physiological differences between these two species may not be reliable characters for species diagnosis. Moreover, discriminating these species in farmed oyster stocks along the west coast has been made difficult by purposeful or unwitting hybridization between the two species in the past.

Molecular markers are now available to screen Kumamoto oyster stocks for purity of breed. Allozymes of isocitrate dehydrogenase, malate dehydrogenase, and mannose phosphate isomerase are diagnostic characters that can be detected in spat. Nucleotide differences in 16S rRNA-coding mtDNA can be used to diagnose

the maternal parent of a single larva. Species-specific oligonucleotide probes have been designed for use in dot blot hybridizations to larval DNA that is enzymatically amplified by the polymerase chain reaction and immobilized on nylon membranes.

**THE USE OF MOLECULAR MARKERS TO ASSESS INBREEDING IN AN INTRODUCED POPULATION OF JAPANESE SCALLOPS, *PATINOPECTEN YESSOENSIS*.** E. G. Boulding,\* John D. G. Boom, and A. T. Beckenbach, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., V5A 1S6, Canada.

The Japanese scallop, *Patinopecten yessoensis*, was introduced to B.C. from Mutsu Bay, Japan between 1983 and 1986 and held and spawned under quarantine conditions; this resulted in the B.C. population being maintained at a small population size for several generations. We are using restriction analysis and direct sequencing of mitochondrial DNA to compare the genetic diversity of the natural, source scallop population with that present in the introduced population.

We are working with mitochondrial DNA as this gives finer resolution than allozyme electrophoresis. Restriction analysis revealed a very high level of genetic diversity in a natural population from Japan relative to what we have seen in other phyla. However the size of the Japanese scallop mitochondria is nearly twice the size of most animal mitochondria so some of the variation we see may not be useful for assessing inbreeding; the extra DNA may be partially composed of tandem repeats which may not be inherited in a simple manner. To separate informative from non-informative variation we are using PCR (polymerase chain reaction) to amplify fast-evolving, protein-coding regions of the mitochondria; we then determine the sequence of the region we amplified. The results from this analysis will enable us to determine whether a severe bottleneck has taken place that might adversely affect economically important traits.

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**GENETIC DIVERSITY IN BRITISH COLUMBIA PACIFIC OYSTER (*CROSSASTREA GIGAS*) POPULATIONS AS DETERMINED BY MITOCHONDRIAL DNA ANALYSIS.** John D. G. Boom,\* E. G. Boulding, and A. T. Beckenbach, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.

To evaluate the population structure and potential of existing *C. gigas* populations to serve as raw material for selective breeding programs, we are measuring levels of genetic diversity in these populations through restriction enzyme analysis of mitochondrial DNA (mtDNA) genotypes.

Substantial variation in several populations has been encountered using this approach. For example, Pipestem Inlet (Barclay

Sound) and Pendrell Sound populations exhibit mean sequence divergences (Nei and Tajima 1981) of .379% ( $\pm .213\%$ ) and .340% ( $\pm .194\%$ ), respectively, and diversities of mtDNA lineages of .822 and .865, respectively. These values are comparable to those determined for the East coast oyster, *Crassostrea virginica*. Furthermore, samples from these two populations can be clearly distinguished on the basis of the mtDNA lineages they contain: while they share 2 lineages in common (and at similar frequencies), the Pipestem and Pendrell samples contain 7 and 8 unique lineages, respectively.

These results suggest that 1) substantial levels of genetic variation exist in these populations; 2) these populations can be clearly distinguished on the basis of mtDNA genotypes; 3) this approach has implications for the management of both hatchery broodstocks and wild populations, and 4) mtDNA analysis provides data pertinent to the study of the reproductive dynamics of this species.

Financial support was provided by grants to A.T.B. the Science Council of British Columbia (#32, SA-2) and the British Columbia Ministry of Agriculture and Fisheries.

**BIOLOGICAL AND ECONOMIC MODELING OF DISEASE PROCESSES IN BIVALVE MOLLUSKS.** Kenneth M. Brooks,\* Pacific Rim Mariculture, 644 Old Eaglemount Road, Port Townsend, WA 98368.

Analytical modeling has been applied to the study of hemic neoplasia in *Mytilus edulis trossulus*. The resulting models, while rather simple in form, provide insights into the relationships between observable parameters associated with disease and mortality in the population. These models fully demonstrate the inappropriateness of predicting mortality at the population level, based solely on the prevalence of some sign of disease. In addition, the models accurately describe growth and mortality and have been used to predict relative biomass as a function of time on a Pacific Northwest mussel farm.

The results of field testing *M. edulis trossulus*, *M. edulis galloprovincialis* and their hybrids in a hemic neoplasia enzootic area are provided and a number of grow-out scenarios described. Significant variations in survival of these populations have been demonstrated and the genetic implications are discussed.

The modeling process is used to develop management recommendations to increase annual production of *M. edulis trossulus* grown in the hemic neoplasia enzootic area of the Pacific Northwest. These recommendations include minimum distances between culture rafts, seed segregation, early harvest times and the removal of all older, diseased mussels from rafts before planting new seed.

This research was conducted at the Battelle Marine Sciences Laboratory with support from the National Cancer Institute, US Army Medical Research and Development Command, US Department of Agriculture and the Northwest College and University Association for Science.

**DISEASE OF JAPANESE SCALLOPS (*PATINOPECTEN YESSOENSIS*) CAUSED BY AN INTRACELLULAR BACTERIUM.** Susan M. Bower\* and Gary R. Meyer, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada, V9R 5K6.

Preliminary grow-out studies on Japanese scallops revealed excellent potential for the culture of this species in British Columbia. Recently, an infectious intracellular bacterium was identified that may account for the poor growth and high mortalities experienced during experimental grow-out in 1989. Laboratory studies indicate that the intracellular bacterium infects the haemocytes and the outcome of the infection is dose dependent. Scallops (7 to 12 cm in shell height) exposed to large numbers of the bacterium via intramuscular inoculation usually die with overwhelming infections between 2 and 4 weeks after injection. These infections are often complicated by septicemias due to other bacteria. Injections of fewer bacteria resulted in a 2 month prepatent period and presence of pinkish-orange pustules up to 10 mm in diameter in the adductor muscle. Histological lesions occurred in the connective tissues of all organs and varied in structure from irregular patches of inflammation, often with a core of necrosis, to encapsulated patches of inflammation that usually contained necrotic cells. These lesions resembled those observed in 63% to 100% of the scallops from 6 grow-out localities that were experiencing poor growth and greater than 75% mortalities in 1989. Laboratory studies to identify the pathogen, develop a method of diagnosis, and to find means of preventing and/or controlling the condition are in progress. However, information on the occurrence and affect of this disease on cultured scallops in the field is required from the aquaculture industry.

**OCEANOGRAPHIC CONDITIONS CONDUCIVE TO THE CULTURE OF THE JAPANESE SCALLOP, *PATINOPECTEN YESSOENSIS*, IN BRITISH COLUMBIA: CRITERIA FOR SITE SELECTION.** Stephen F. Cross,\* Aquamatrix Research Ltd., 204-2527 Beacon Ave., Sidney, British Columbia, Canada V8L 1Y1.

The waters of Little Espinosa Inlet, located off the northwest coast of Vancouver Island, have demonstrated the highest grow rates and the best survival of hatchery produced Japanese scallop seed (1–3 cm) in British Columbia to date. This paper describes the spatial and temporal changes in the water column properties of this unique oceanographic system, and discusses these conditions in terms of scallop growth and survival. A synopsis of important site selection criteria, based on the 2½ year evaluation of our site, is also provided.

Using seed produced at the Pacific Biological Station (DFO), scallop growth (shell height) and survival were monitored monthly over a 2½ year period. Animals grown in Pearl and Lantern nets were deployed in a stratified design at depths of 5, 10, 15, 20, and 25 metres (3 replicates/depth), with water temperature, salinity,

and dissolved oxygen measured concurrent with the scallop growth and survival estimates.

It was found that site bathymetry plays an important role in maintaining water column stability and in ensuring that an optimum salinity and temperature environment is provided throughout the year at this site. Cold water entering over an outer sill (inlet mouth) during flood tides replenishes the lower water column water, while warmer water is withdrawn from the inlet across the surface during the ebb tide. At mid-summer, the warmer surface water extends as low as 5 metres (the upper zone in which our scallops were grown). Although plankton availability increases during this period, growth is retarded once water temperature reaches 12.5°C. When temperatures exceed 15°C, mortality increases.

The sensitivity of the Japanese scallop to water column instability, particularly with respect to salinity and temperature, makes proper site selection essential for this species. Our success with the culture of this species is directly related to the oceanographic conditions of the site.

**EVIDENCE SUPPORTING A TERMINAL MOLT IN THE SPIDER CRAB, *LOXORHYNCHUS GRANDIS*.**Carolynn S. Culver\* and Armand M. Kuris, Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, California 93106.

In Canada and the west coast of the United States, spider crabs support valuable fisheries. Crabs of the family Majidae have been characterized as exhibiting a terminal molt in both sexes. However, this traditional belief has not been experimentally proven for most majid species. With a recent debate over the existence of a terminal molt in male *Chionoecetes* spp., it now becomes important to verify the occurrence of a final molt in male members of other spider crab species.

Currently such information is needed for the sheep crab, *Loxorhynchus grandis*. This majid crab is being utilized along the south-central coast of California for two different types of markets; a whole body, live market and a crab claw market. The following data were used to evaluate the existence of a terminal molt in *L. grandis*: 1) Relative growth analysis identified three distinct groups of males; small and large juveniles and morphometrically adult animals. The wide adult size range can be explained by the juvenile size range and associated molt increments; 2) Experimentation using induced autotomy resulted in molting activity of large juvenile animals, but not of adults; 3) Development of a premolt cuticle has only been seen in juvenile crabs, never in adult crabs, regardless of size or carapace condition; 4) The breakage plane of autotomized limbs become calcified in adults, not permitting regeneration. In contrast, juveniles develop a soft, leathery membrane which enables regeneration to occur; and 5) Only adults undergo substantial carapace abrasion and obtain large encrusting organisms. These experimental results and field observations sup-

port the hypothesis that sheep crabs cease to molt after attaining the morphometric molt of puberty.

**GROWTH OF THE JUVENILE PACIFIC OYSTER, *CRASSOSTREA GIGAS* (THUNBERG), IN EFFLUENT WATER FROM A SALMON AND RED MACROALGAL POLY-CULTURE SYSTEM.** Maria E. Diaz,\* W. McNeil, and C. E. Levin, Department of Fisheries and Wildlife, Oregon State University, Hatfield Marine Science Center, Newport, Oregon 97365.

Experiments conducted in the fall of 1990 and spring of 1991 determined the growth rates of 5 mm spat of *Crassostrea gigas* cultured in four different treatment effluent from tank cultured salmon, salmon tank effluent stocked with the red macroalga, *Palmaria mollis*, ambient sea water enriched with *P. mollis* and ambient sea water alone.

In the fall, fish effluent and *P. mollis* effluent treatments provided significantly higher growth than the ambient sea water. However, in the spring, growth was significantly greater in ambient sea water than in any of the other treatments. Possible causes for these variations are discussed.

**HYBRIDS AMONG THE PACIFIC, AMERICAN AND THE SUMINOE OYSTERS: INDUCTION AND AQUACULTURE POTENTIAL.** Sandra L. Downing,\* School of Fisheries, WH-10, University of Washington, Seattle, WA 98195.

Hybrids are used by other agricultural industries to improve the marketed product. With pollution and disease decimating the populations of the American oyster, *Crassostrea virginica*, a hybrid with the hardier Pacific oyster, *Crassostrea gigas*, could be one answer for revitalizing the east coast industry. In addition, the relative success of different hybrid crosses indicate how close the species are phylogenetically.

Using full factorial designs, crosses were made between *C. gigas* and either *C. virginica* or *C. rivularis*, the Suminoe oyster. The Pacific and the Suminoe oysters were crossed successfully and yielded spat. While initial survival for the American and Pacific crosses were at least 10%, the larvae generally failed to grow to the umbo larval stage (>100  $\mu\text{m}$ ). The few surviving outliers suggested contamination and stress the importance of hybrid confirmation which will be discussed. Inducing triploidy into either hybrid cross did not increase survival.

The *C. gigas* (female) by *C. rivularis* (male) survived the best. It was fertile and a second generation of both diploid and triploid offspring were produced. The aquaculture potential of the inter-specific and monospecific crosses will be discussed.

**EARLY DETECTION OF *ALEXANDRIUM CATENELLA* BY AN IMMUNOCHEMICAL AND FLOW CYTOMETRIC METHOD.** L. B. Eisner,\* M. J. Perry, and M. C. Talbot, School of Oceanography, University of Washington, Seattle, Washington 98195.

A sensitive immunological method has been developed for the

early detection of the toxic marine dinoflagellate *Alexandrium catenella*. Monoclonal antibody technology is utilized to produce a fluorescently-labeled antibody to cell walls of *A. catenella*. This labeled antibody allows *A. catenella* cells to be detected and identified in seawater samples. Cell walls labeled with this method can be visually distinguished by epifluorescent microscopy or counted using a flow cytometer. Flow cytometry allows a large number of samples to be counted rapidly. Flow cytometric samples measured on consecutive days can be used to calculate growth rates. Cells can be identified at very low numbers, i.e. before they grow to concentrations that can affect fisheries. Further screening and field testing of the current antibody is to be conducted. This method can be used to detect other harmful phytoplankton species.

**LESLIE ANALYSIS OF LOGBOOKS FROM THE DIVE FISHERY FOR ABALONE, *HALIOTIS KAMTSCHATKANA*, IN BRITISH COLUMBIA, 1977–1990.** Robert W. Elner\* and Alan Campbell, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, V9R 5K6.

Commercial logbook data from the dive fishery for abalone in British Columbia were analyzed by the Leslie method. The method involves regressing a measure of fishing success against cumulative captures through a fishing season. The intercept of the regression on the cumulative catch axis gives an estimate of the total available biomass of commercial sized abalone, and the slope is an estimate of catchability. Estimates of exploitation rate, stock size at the start and end of the season and interannual recruitment were derived. Trends in annual commercial biomass levels were similar for the nine Statistical Areas during 1977–1990. Biomass estimates for the late 1980's were a fraction of those for the late 1970's although, in most cases, mean annual catch per diver hour remained stable. Generally, annual recruitment to the fishable stocks appeared less than landings except during the early-to-mid 1980's when the estimated stock sizes increased. The applicability and potential of the Leslie method as an assessment tool for dive fisheries are discussed.

**STUDIES ON NUCLEAR INCLUSION X (NIX) IN THE PACIFIC RAZOR CLAM, *SILIQUA PATULA*.** Ralph Elston and Ann Drum, Battelle Marine Sciences Laboratory, 439 West Sequim Bay Road, Sequim, WA 98392; Arthur Gee and David Kerk, Department of Biology, Pacific Lutheran University, Tacoma, WA 98447.

Nuclear inclusion X (NIX) was discovered in 1983 in Washington state populations of the Pacific razor clam and was associated with massive mortalities of the clam. NIX is structurally prokaryotic and resides in non-ciliated branchial epithelium of the clams. Resilient host nuclei swell up to 25  $\mu\text{m}$  in diameter as the pathogen develops. Two mid-Washington beaches show characteristically high average infection intensities and four important clam beaches in Washington show recurrent annual and relational cycles of average infection intensity.

A biopsy method has been devised and evaluated for ongoing studies of NIX infection intensities in natural and experimental populations of clams. The status of time series microcosm studies with varied environmental parameters using biopsy examination to observe NIX development will be reported.

Following development of a method to physically isolate NIX, 16S ribosomal RNA sequence studies to more precisely identify the disease causing organism were undertaken. These studies indicated that NIX is a member of the gamma subdivision of the *Proteobacteria*. Determination of the three highly variable sequence regions of this gene is also the basis for an environmental and tissue probe for NIX, now under development.

Progress on studies to characterize the immunocompetence of individual clams, in reference to branchial lesions identified at biopsy, utilizing a bacterial clearance assay, will be reported.

Research supported by the Washington State Department of Fisheries.

**FIRST OCCUPANT 0+ DUNGENESS CRAB IN INTERTIDAL SHELL HABITAT INHIBIT STRONG RECRUITMENT OF SUBSEQUENT COHORTS.** Miriam Fernandez, Oscar Iribarne, and David Armstrong, School of Fisheries WH-10, University of Washington, Seattle, WA 98195, USA.

Megalopae of Dungeness crab *Cancer magister* Dana arrived in the Grays Harbor Estuary (WA) and settled in successive pulses during the months of May and June, 1991. Field and laboratory experiments were conducted to examine interaction between cohorts of juvenile benthic instars that settle in the intertidal zone.

The first cohort of megalopae entered the estuary between May 15–20 and settled in ("occupied") oyster shell habitat previously constructed in March 1991, at densities ranging from 155 to 196 first instar (J1) crab  $\cdot m^{-2}$ . Subsequently, a second phase of shell habitat was constructed that did not contain crab of the first pulse ("unoccupied"). Between June 15 and 18, a second cohort of megalopae entered the estuary and settled in both "occupied" (instars by then were J2 and J3) and "unoccupied" shell habitat where the respective ranges of J1 crab densities were 9–37 and 168–298  $\cdot m^{-2}$ . Laboratory experiments indicated that megalopae select shell over "open" space to settle and metamorphose, but make no distinction between new (clean) or aged (fouled) shell. However, older J1 (>48h post-settlement) and J2 and J3 instars are predators of megalopae and newly molted J1. These results suggest several hypotheses to account for much lower density of second cohort J1 in the "occupied" vs "unoccupied" shell: 1) older instars cannibalize newly settled recruits of the same age class; 2) intrinsic carrying capacity of the shell habitat was generally saturated by the first cohort leaving little space for the second; and 3) megalopae of the second cohort "detected" cannibalistic conspecifics in the "occupied" shell and so generally avoided that habitat. We feel that hypothesis No. 1 is best supported by these data, and conclude that cannibalism by small instars days or

weeks different in age can substantially reduce survival of later cohorts that settle in shell habitat.

**HAPLOSPORIDAN INFECTIONS OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* THUNBERG, FROM JAPAN AND CALIFORNIA, U.S.A.** Carolyn S. Friedman\*, California Department of Fish and Game, Fish Disease Laboratory, 2111 Nimbus Rd., Rancho Cordova, California 95670; Ronald P. Hedrick, Department of Medicine, School of Veterinary Medicine, University of California, Davis, California 95616.

Haplosporidan parasites were observed in Pacific oysters, *Crassostrea gigas*, from Matsushima Bay, Japan during a health examination prior to being imported into California waters in 1989 and 1990 (Friedman et al. 1991, In press, J. Invertebr. Pathol.). Host response to and morphology and tissue specificity of the parasite stages closely resembled those of *Haplosporidium nelsoni*, the causative agent of Delaware Bay Disease or "MSX" of the American oyster, *C. virginica*.

In response to these observations, oysters from Matsushima Bay, Japan were not imported into California. We sampled two embayments in California, Drakes Estero, the bay that historically received these oysters, and Tomales Bay, an adjacent embayment where Pacific oysters are cultured. All oysters appeared healthy upon gross examination. Three percent of the Pacific oysters sampled in March from Drakes Estero had mild localized to severe systemic plasmodial infections, while only 1% of the Pacific oysters sampled in May contained haplosporidan infections. An inflammatory response to the haplosporidan infections was observed in the March samples. Infected animals from the May sample primarily contained haplosporidan plasmodia within the epithelium of the heart that did not elicit a host inflammatory response. One oyster from this sample had plasmodia within the stomach epithelium that elicited a mild inflammatory response. No haplosporidan parasites were observed during the September survey of Tomales Bay.

**ESSENTIAL FATTY ACID PROFILES AND SIZES OF ELEVEN SPECIES OF PHYTOPLANKTON COMMONLY USED IN CRUSTACEAN HATCHERIES.** N. G. Ginther, L. D. Townsend,\* and J. N. C. Whyte, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., V9R 5K6.

Eleven species of unicellular phytoplankton, five from the class Bacillariophyceae (one pennate, four centric diatoms), one species of Cryptophyceae, one species of Eustigmatophyceae, two species of Prasinophyceae and two species of Prymnesiophyceae were chosen for study on the basis of their relevance to shellfish nutrition. The phytoplankton were grown in semi-continuous culture to simulate hatchery conditions. Each species was analyzed with regard to fatty acid profile and size. Species within a class and between classes were compared.

Two fatty acids, eicosapentaenoic acid (20:5n3) and docosa-

hexaenoic acid (22:6n3), both considered as essential fatty acids for marine shellfish, were specifically profiles as a percentage of total fatty acid composition. The highest percentage of 20:5n3, (32.9%), was found in a *Nannochloropsis* species of the class Eustigmatophyceae. As a class, Bacillariophyceae generally contained high levels. All phytoplankton analysed had 10% or less 22:6n3 fatty acid, the highest level, being found in the prymnesiophyte, *Isochrysis galbana*. Each species was sized by cell diameter and volume. The cell diameter ranged from the eustigmatophyte, *Nannochloropsis* sp. at 2.0 microns to the cryptophyte, *Rhodomonas lens*, at 11.7 microns. The value for bivalve nutrition, with respect to fatty acid profile and cell size, is discussed. Results are compared with other investigations.

**RESEARCH INTO MANILA CLAM CULTURE IN BRITISH COLUMBIA.** William A. Heath,\* Fisheries and Food, Aquaculture and Commercial Fisheries Branch, 2500 Cliffe Avenue, Courtenay, B.C. V9N 5M6.

Commercial culture of the Manila clam, *Tapes philippinarum*, is advancing rapidly in British Columbia, with over 25 companies operating more than 50 sites. Research projects are being conducted on hatchery, nursery and growout phases of clam culture. Ministry studies of Manila clam growout are comparing growth and survival of outplanted seed (6–8 mm) through to harvest size (ϕ38 mm); various treatment combinations of seeding density, netting cover, tidal height and substrate characteristics are being used on experimental plots at a range of beach sites in southern British Columbia. Results from the first complete year of sampling and the implications on the economics of Manila clam culture in B.C. will be presented.

**GENETIC AND ENVIRONMENTAL COMPONENTS OF VARIANCE IN HARVEST BODY SIZE AMONG PEDIGREED PACIFIC OYSTERS *CRASSOSTREA GIGAS* FROM CONTROLLED CROSSES.** Dennis Hedgecock,\* Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923; Ken Cooper, Coast Oyster Co., Quilcene, WA 98376; and William Hershberger, School of Fisheries, University of Washington, Seattle, WA 98195.

A collaborative project of the USDA's Western Regional Aquaculture Consortium made eight, 8 male × 3 female experimental crosses of Pacific oysters, two factorial and six hierarchical, yielding a total of nearly 192 families. Progeny were set on cultch, randomized on long lines and deployed to growout areas in Puget Sound, WA, and Humboldt Bay, CA. Wet body (meat) weights at commercial harvest sizes (14 to 40 mos. age) have been obtained for 12 of 22 cross-locality combinations. On the basis of data so far analyzed, there is substantial phenotypic variation in body size (C.V.'s are ~30% of mean weight). In one cross, full-sibs grown on different cultch varied significantly in body size, possibly as the result of differences in density, but this cultch effect was not observed in other crosses. Sex of progeny also

contributes significantly to variation in body size, females being ~10% heavier than males. Components of variance in body size associated with sire, dam, dam-within-sire (hierarchical crosses), and sire-by-dam interaction (factorial crosses) are significant in most cases.

These preliminary results suggest that there is substantial additive genetic variation, moderate heritability (~0.2) and a reasonable expectation for response to selection for harvest body size in Pacific oysters. Selection programs may also need to take into account non-additive genetic components of variance. Estimates of genetic components of variance and heritability from any one cross are fairly imprecise, but consistency over the set of crosses increases confidence in these general conclusions.

**INTENSIVE SANITARY SURVEY METHOD USING TRACER DYES.** Linda Hofstad,\* Cathlene Hansen, and William Fiorilli, Thurston County Public Health and Social Services Department, Resource Protection Section, Olympia, Washington 98502.

An intensive sanitary survey procedure was developed and implemented to find sources of nonpoint pollution from on-site septic systems. This survey technique was used successfully along marine shorelines in commercial shellfish growing areas of Thurston County. Although two marine sampling stations had failed to meet water quality standards for years, various study efforts had been relatively unsuccessful in pinpointing the nonpoint sources.

Staff theorized that though few if any obvious surfacing on-site septic system failures were occurring, probably numerous systems were leaching varying amounts of partially treated sewage into the marine waters via bulkhead drains, seeps on bluffs, seeps and springs on beaches as well as intertidally.

In order to test our hypothesis we developed an intensive survey technique utilizing fluorescein dye tracing. Because fluorescein dye is adsorbed onto charcoal, packets of coconut charcoal were placed in suspected wastewater pathways at each survey site. At one and two week intervals the packets were retrieved. After thorough washing, the charcoal was emptied into a glass container and a solution of KOH was poured over. This elutant sample was sent to Ozark Underground Laboratory and analyzed by a Shimadzu scanning spectrofluorimeter for presence of dye.

Dye was found to be present in about 60% of all sites where packets were placed. Prior sanitary survey techniques would have yielded a 2.5% rate of failure.

**MEGALOPAL SPATIAL DISTRIBUTION AND STOCK SEPARATION IN DUNGENESS CRAB.** G. S. Jamieson and A. C. Phillips, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6.

The day-time depth ranges of *Cancer magister* megalopae from the outer coast and from Georgia Strait differ significantly, with megalopae at 25 and 160 m depth, respectively. At night, both populations of megalopae are mostly in the top metre of the water

column. Juan de Fuca Strait, which connects Georgia Strait to the Pacific Ocean, typically has an estuarine circulation, with outflow in the top 50–100 m and inflow at deeper depths. With a daylight:dark ratio in the spring and summer (when megalopae are present) of about 3:1, the consequence is that Georgia Strait and outer coast megalopae are mostly retained within their own oceanographic systems. Small, occasional intrusions of outer coast megalopae into the Strait may occur when estuarine flow in Juan de Fuca Strait temporarily breaks down following sustained strong southwesterly winds. However, such intrusions are typically restricted to the southern side and head of Juan de Fuca Strait and do not penetrate far into Georgia Strait.

Cold (<10°C) deep water in Georgia Strait is suggested to be the explanation for both the delay in seasonal timing of larval crab settlement and a smaller physical size at settlement of Georgia Strait megalopae. These differences allow outer coast and Strait megalopae to be readily separated in survey samples.

**SOME PHYSIOLOGICAL EFFECTS OF A CHRONICALLY IMPLANTED CATHETER IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*.** Trevor O. Jones,\* J. D. Morgan, and G. K. Iwama, Department of Animal Science, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4.

A cannulation technique that provides a method for non-lethal collection of hemolymph from oysters will be presented. Cannulation techniques allow for repetitive sampling *in-vivo* without having to handle or disturb animals. Fewer animals are required and a more representative description of physiological processes under resting conditions can be obtained.

*In-vivo* measurements of hemolymph pH, salinity, total cell count and PO<sub>2</sub> were performed on both cannulated and uncannulated control animals. *In-vitro* analysis of phagocytic activity of representative hemocytes; using fluorescent latex beads, from cannulated and control animals was also performed.

Hemolymph was sampled at time 0, 4, 8, 12, 24 and 120 hours. Cannulated animals exhibited a significant ( $p < 0.05$ ) increase in total blood cell count at 120 hours. Hemolymph PO<sub>2</sub> values of cannulated animals were significantly ( $p < 0.05$ ) greater at 12 and 24 hours when compared to controls. The mean % adherence of fluorescent latex beads to hemocytes was significantly ( $p < 0.05$ ) greater for cannulated animals at 8, 12, 24, and 120 hour intervals when compared to controls.

**DEVELOPMENT OF PARTICLE FILTRATION IN EARLY JUVENILE JAPANESE SCALLOPS (*PATINOPECTEN YESOENSIS*).** Brian C. Kingzett,\* Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6 and N. Bourne, Dept. of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

Filtration rates of juvenile Japanese scallops (postmetamorphic to approximately 2 mm in shell height) were determined using various species of phytoplankton. Scallops were contained within

a series of miniature flow-through chambers and filtration rates measured for phytoplankton concentrations of  $1-5 \cdot 10^4$  cells  $\cdot$  ml<sup>-1</sup>. A Coulter Counter Multisizer II was used for particle size and concentration analysis.

Filtration rates increased with phytoplankton concentration until a maximum cell density was reached. Differences in filtration rates for various species of phytoplankton indicate that efficiency of the ctenidia (gill) may be related to factors other than particle size. The development of the ctenidia may be correlated with increases in overall filtration ability. Knowledge of size and density-related feeding rates are discussed in relation to the development of feeding regimes in nursery culture.

**EFFECT OF CAPSULE COMPOSITION ON THE DELIVERY OF DIETARY PROTEIN TO THE MUSSEL, *MYTILUS TROSSULUS*.** Daniel A. Kreeger\* and Christopher J. Langdon, Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365.

The effect of protein source on the mussel's (*Mytilus trossulus*) ability to utilize protein microcapsules (PM) was studied. PM were prepared with protein derived from either crab or microalgae and each was spiked with <5% w/w <sup>14</sup>C-labeled algal protein. Crab and algal PM were each incubated for 24 h with either amylase, trypsin, protease, or extracts from the style or diverticula of *M. trossulus*. Significantly ( $p < 0.05$ ) more [<sup>14</sup>C]protein was released from crab PM than from algal PM in all enzyme treatments except trypsin. Mussels were fed <sup>14</sup>C-labeled crab or algal PM, and digestion and assimilation efficiencies for [<sup>14</sup>C]protein from crab PM (37%, 26%, respectively) were significantly ( $p < 0.05$ ) greater than those from algal PM (25%, 21%, respectively).

In addition, two different carbohydrates, maltodextrin and amylose, were added at 33% w/w to crab PM, and their *in vitro* and *in vivo* digestibilities compared to those of 100% crab PM. Treatment by amylase, trypsin, and mussel style extract released significantly ( $p < 0.05$ ) greater [<sup>14</sup>C]protein from 100% PM than from PM supplemented with either maltodextrin or amylose. In contrast to the *in vitro* results, mussels showed no significant differences in their ability to digest and assimilate [<sup>14</sup>C]protein from amylose-supplemented PM (40% and 8%, respectively), maltodextrin-supplemented PM (32% and 6%, respectively), or 100% PM (27% and 6%, respectively).

**SURFACE PROPERTIES OF PARTICLES EFFECT THEIR SELECTION BY CLAM LARVAE (*MERCENARIA MERCENARIA*).** C. J. Langdon and V. L. Shaffer, Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365; S. M. Gallager, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543; D. K. Stoecker, Horn Point Environmental Laboratories, University of Maryland, Cambridge, Maryland 21613.

Selection of captured particles at the mouth and oesophagus of 3 day old clam (*Mercenaria mercenaria*) larvae was observed by

video microscopy when larvae were fed on either uncoated glass beads (2.1  $\mu\text{m}$  diameter) or organosilane-coated glass beads. Uncoated glass beads were rejected at the mouth and oesophagus of larvae at significantly higher probabilities than coated beads, resulting in a significantly higher probability of acceptance of coated beads into the stomach.

The feeding history of larvae affected bead rejection. Larvae that were pre-fed for about 1 hour on the alga *Isochrysis galbana* (ISO) rejected significantly higher proportions of both bead types at the mouth and, overall, accepted a significantly lower proportion of both bead types into the stomach than prestarved larvae.

When pre-starved larvae were fed on either uncoated or coated glass beads in combination with ISO, larvae rejected a significantly higher proportion of uncoated beads at the oesophagus compared with coated beads; whereas both beads types were rejected with equal probability at the mouth. Similarly, uncoated beads significantly increased rejection of algae at the oesophagus compared with coated beads, but there was no effect of bead type on rejection of algae at the mouth. The oesophagus of clam larvae appears to be more important than the mouth in selection of particles.

**GENETIC CHARACTERISTICS AND RELATIONSHIPS OF FIVE RAZOR CLAM (*SILIQUA PATULA*) POPULATIONS ALONG THE PACIFIC COAST OF NORTH AMERICA.** Larry L. Leclair\* and Stevan R. Phelps, Genetics Unit, Washington Department of Fisheries, Room 115 General Administration Building, Olympia, Washington 98504.

We electrophoretically examined razor clams (*Siliqua patula*) from five intertidal locations along the Pacific coasts of Alaska, British Columbia, Washington, and Oregon, to determine the level of genetic variation within the species and among regions. All five locations exhibited high levels of within population genetic diversity. The average heterozygosity ranged from 0.25 to 0.27 and the mean number of alleles per locus was 3.4 to 4.6, based on 24 loci. We found no reduction of genetic diversity within the Washington populations compared to populations that had not experienced large mortalities due to the pathogen, nuclear inclusion X (NIX).

The genetic diversity among regions is low, and alleles that occurred at a frequency of 0.05 or greater in one collection, were generally observed in all other collections. However, we found significant differences in allele frequencies among collections. The most genetically similar populations were the two from Washington, while the greatest genetic differences occurred when the two Washington collections were combined and compared to the Alaska sample. Differences in the susceptibility of razor clams to NIX among locations may have a genetic basis.

**PREDATOR PREY INTERACTIONS BETWEEN JUVENILE GEODUCK CLAMS (*PANOPE ABRUPTA*) AND FIVE ASSOCIATED PREDATORS IN PUGET SOUND, WASHINGTON.** Amy Leitman and Brady Blake, WA Department of Fisheries, Point Whitney Shellfish Laboratory, Brinnon, Washington 98320.

Over the last decade, dozens of field trials using hatchery raised geoduck seed were done to evaluate the feasibility of enhancing natural stocks of geoduck clams. To clarify the nature of predator/prey interactions found in the field, a laboratory experiment was done comparing the extent of predation by five predator species (*Cancer productus*, *C. gracilis*, *Pisaster brevispinus*, *Polinices lewisii*, and *Lepidopsetta bilineata*) on four sizes of geoduck seed. The seed size preference of each individual predator species was determined and predation rates of each predator were compared.

Four of the five predators seemed to selectively consume geoduck seed of different sizes. Rates of predation on the two largest seed sizes (12 mm and 16 mm) varied significantly among almost all predators, whereas predation rates on the smallest seed sizes (4 mm and 8 mm) varied only slightly and were not statistically significant. *C. productus* consumed more geoduck seed than the other predator species.

An inventory of predator specific and seed size specific predation rates on geoduck seed can be used to develop a seed planting strategy that optimizes geoduck enhancement activities. Greater survivorship of planted geoduck seed is likely if predation patterns can be predicted and avoided.

**LAND-BASED POLYCULTURE OF SALMONIDS, MACROALGAE, AND CLAMS.** John-Eric Levin\* and William J. McNeil, Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365.

Winter (November–February) growth rates of juvenile (2 mm) Manila clams (*Tapes japonica*) grown in raceways were significantly higher in effluents from tank cultured coho salmon (*Oncorhynchus kisutch*) after passage of effluents through tank cultured commercially valuable red marine macroalgae (*Palmaria mollis*) than in salmon effluent alone or in ambient seawater.

Maximum growth of juvenile (2–5 mm) *T. japonica* grown in upwellers receiving fish/macroalgae effluent during spring (March–May) occurred at a flow of 13 l min<sup>-1</sup> per kg clam biomass. Stocking density of macroalgae was 28 g per g of clam biomass.

Stocking density relationships of fish, macroalgae, and clams, as well as economic projections for a land-based facility for the culture of these organisms will be discussed.

**BURROWING SHRIMP, (*CALLIANASSA CALIFORNIENSIS*, *UPOGEBIA PUGETTENSIS*): FISHERIES MANAGEMENT AND GRAY WHALE, (*ESCHRICHTIUS ROBUSTUS*) PREDATION RESEARCH.** Jay G. Odell\* and W. A. Wood, Washington State Department of Fisheries, 1000 Point Whitney Road, Brinnon, Washington 98320.

Ghost shrimp (*Callianassa californiensis*) and mud shrimp (*Upogebia pugettensis*) are the targets of a new bait fishery managed by the Washington State Department of Fisheries and the Washington State Department of Natural Resources. Life history information and management techniques are presented.

One of the concerns of burrowing shrimp fisheries management is to determine whether there are potential conflicts between the commercial fishery and gray whales (*Eschrichtius robustus*). Gray whales are significant ghost shrimp predators. Feeding whales create pits which can be detected from the air. Aerial surveys were conducted to assess the extent of gray whale feeding on ghost shrimp. Shorelines of Saratoga Passage and Port Susan in Puget Sound, Washington were videotaped at low tide to document the number and location of gray whale feeding pits. Whale feeding activity has increased dramatically since 1990. Sampling has been conducted to determine the average number and weight of shrimp removed from a feeding pit. Biomass consumed by whales on commercial harvest tracts was comparable to amounts taken by the commercial fishery. In the study area as a whole, biomass consumed by whales was over 1000% more than that taken by the fishery.

**EFFECTS OF DIETARY ALGAL AND LIPID SUPPLEMENTS ON GONADAL AND LARVAL DEVELOPMENT OF *CRASSOSTREA GIGAS KUMAMOTO* (THUNBERG).** Anja M. Robinson, Hatfield Marine Science Center, Department of Fisheries and Wildlife, Oregon State University, Newport, Oregon 97365.

Larval survival and development during hatchery rearing depends not only on hatchery culture techniques but also on the quality of eggs at the time of spawning. The broodstock conditioning environment can be manipulated to optimize the stage of development and quality of eggs at spawning.

In this study broodstock oysters were fed on algal and lipid supplements during the conditioning for spawning. When conditioning was initiated early in the season (March to early April) both the proportion of fertilized eggs that successfully developed into straight-hinged larvae and spat were higher in progeny derived from algal or lipid supplemented broodstock compared to those obtained from non-fed oysters.

Although feeding regime had no effect on the fatty acid composition of oysters, it did affect that fatty acid composition of the eggs. The w3 fatty acid content was significantly higher in eggs released from algal-supplemented broodstock oysters than in eggs from lipid-supplemented or non-fed oysters. Enrichment of the w3 fatty acid composition of the eggs from algal-supplemented oysters may explain the success rate in egg development and metamorphosis.

**COMMERCIAL CULTIVATION OF THE JAPANESE SCALLOP, *PATINOPECTEN YESSOENSIS*, IN BRITISH COLUMBIA.** Robert G. Saunders,\* Island Scallops Ltd., R.R. #3, Site 327, C-1, Qualicum Beach, B.C. V0R 1B0 and Neil F. Bourne, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.

In the 1980's, a research program at the Department of Fisheries and Oceans Pacific Biological Station developed hatchery and nursery technology for producing juveniles of the imported Japanese scallop, *Patinopecten yessoensis*. Based on the results of

this experimental work, a private company, Island Scallops Ltd., built a hatchery for rearing the Japanese scallop in 1989 and began operations in 1990.

Japanese scallops are spawned in the hatchery from January until July. The larvae are reared in large tanks until metamorphosis, a period of approximately three weeks. Mature larvae are set on monofilament netting stuffed in mesh bags, reared in the hatchery for 2–4 weeks, then transferred to an ocean nursery for 3–4 months. For intermediate grow-out, the juveniles are transferred to pearl nets and suspended from submerged longlines. Final grow-out takes place by "carhanging" the scallops until they reach market size, about 10 cm. The entire process from spawning to marketing is anticipated to take from 18 months to 2 years.

**SURVIVAL AND CAPTURE OF PACIFIC RAZOR CLAM ON TWO WASHINGTON BEACHES.** J. Warren Schlechte,\* Steven G. Smith, Annette Hoffmann, and John R. Skalski, Center for Quantitative Science, University of Washington, Seattle, WA 98195.

Monthly survival rates and capture probabilities for the Pacific Razor clam (*Siliqua patula*) were investigated at two beaches along the Washington Coast using tag-release methodology. One plot was established at each beach. Three sites within each plot were allocated according to tidal height.

Analysis of the data was performed using SURPH (SURvival under Proportional Hazards), a survival model which utilizes maximum likelihood theory for tag-release data with population specific covariates. Maximum likelihood estimates for survival rates were computed for a variety of effects. Analysis of deviance and likelihood ratio tests showed significant differences among survival rates. These differences were attributable to monthly (period) effects, to beach level effects, and to handling induced mortality effects. Estimates of monthly survival rates ranged from a high of 1.000 in January to a low of 0.6364 in August.

Capture probabilities for each beach were also estimated. Capture probabilities were highly variable. The differences were attributable to monthly (period) effects, to beach level effects, and to biopsy effects.

Further analysis will continue, to examine the effects of individual clam covariates on survival. Covariates of interest include, but are not limited to, length and NIX infection intensity.

**MUSSEL CULTURE IN BRITISH COLUMBIA: THE INFLUENCE OF SALMON FARMS ON MUSSEL GROWTH.** B. Taylor, G. Jamieson, and T. Carefoot, Department of Zoology, University of British Columbia, Vancouver, B.C., V6T 1Z4.

Use of salmon farms as sites for mussel culture was investigated with respect to possible advantages of nutritional enrichment. Mussels were cultured at different distances around two salmon farms. Three growth parameters: condition index, carbohydrate content, and crude protein content were monitored at 3–6 wk intervals from September 1988–August 1989. Distinct seasonal differences were observed in all parameters, but distance

from the farm did not substantially influence mussel growth, nor did the farms increase available food for mussels. Measures of seston and chlorophyll concentration, made concurrently with mussel collections, indicated that neither a direct contribution of nutrients in the form of fish feed and faeces, nor an indirect contribution of waste nitrogen to augment phytoplankton production, occurred. This was despite currents flowing at least part of the time in a direction from the farms to the mussels.

**EFFECTS OF MONOSPECIFIC ALGAL DIETS OF VARYING BIOCHEMICAL COMPOSITION ON THE GROWTH AND SURVIVAL OF *CRASSOSTREA GIGAS* LARVAE.** Peter A. Thompson\* and Paul J. Harrison, Dept. of Oceanography, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada.

By growing the marine diatom, *Thalassiosira pseudonana*, in steady-state turbidostats or chemostats under different conditions of light, nutrient status and temperature, it was possible to produce cells varying in their biochemical composition. Variation in the phytoplankton's carbon, nitrogen, protein, lipid, carbohydrate, and fatty acid composition was examined for its influence on the growth and survival of larval *C. gigas*.

Growth rates of the oyster larvae fed cells high in carbon, carbohydrate and the short chain saturated fatty acids 14:0 and 16:0 were repeatedly better food items for these animals. We conclude that high energy *T. pseudonana* cells grown under high light and nutrient saturation are a superior diet for *C. gigas* larvae.

The fatty acid composition of the oyster larvae may be useful predictors of the oysters' growth rate. Correlation analysis indicates the oysters' dietary source of the nonmethylene interrupted fatty acid 22:2j may be 16:1ω7.

**THE EFFECTS OF SUBSTRATE MODIFICATION ON THE GROWTH AND SURVIVAL OF PLANTED MANILA CLAMS, *VENERUPIS JAPONICA*.** Derrick R. Toba,\* University of Washington, School of Fisheries WH-10, Seattle, WA, 98195; Doug Thompson, Washington Department of Fisheries, Point Whitney Shellfish Lab, 1000 Point Whitney Road, Brinnon, WA, 98320; Kenneth K. Chew, University of Washington, School of Fisheries WH-10, Seattle, WA 98195.

Bywater Bay on Hood Canal was originally gravelled by the Washington Department of Fisheries in 1983 to increase natural clam production. In 1988, it was well below the target of 10,000 pounds per acre. It was thought that gravel compaction and predation were the two main causes of low clam productivity at this site. The existing gravel plot was rototilled to a depth of 10 cm alone and the addition of crushed oyster shell, tilled into the substrate to a depth of 10 cm were tested to alleviate the impacts of gravel compaction. Crushed oyster shell helps to stabilize the substrate and provide interstitial space. Predator netting was also used on half of each plot to limit the effects of predation. The plots were seeded with 6-8 mm Manila clams.

Initial results have indicated that there is higher survival on the half of the plot protected with predator protection netting. There was little difference between the survival rates of the different treatments. Growth, on the other hand, was greater for plots that were rototilled only and had crushed oyster shell added than for the control.

**CRABBY PREDATORS IN OYSTER TRAYS.** Sylvia Behrens Yamada\* and Heidi Metcalf, Zoology Department, Oregon State University, Cordley Hall 3029, Corvallis, OR 97331-2114.

The Oregon cancer crab, *Cancer oregonensis*, is a major predator of sub-market size oysters. Of all the crabs that settle inside oyster trays, this species has the most powerful claws for its size, and thus is equipped to do the most damage to growing oysters. Unlike its cousins, the Dungeness and red rock crabs, *Cancer oregonensis* attains a maximum carapace width of only 45 mm. This unassuming size has caused some growers to underestimate the predatory ability of this species. Oyster farms on San Juan, WA and Lasquety Island, B.C. report estimated mortality rates on young oysters exceeding 40%. Losses are particularly high during the winter when crabs are growing faster than oysters.

The aim of our research is: 1) to determine the maximum size at which Pacific oysters are vulnerable to different size classes of *Cancer oregonensis*, 2) to evaluate the feeding rates of various size crabs on standard size oysters, and 3) to determine the crab size above which oyster growers should start removing crabs.

The largest *Cancer oregonensis* is able to feed on oysters up to 60 mm in length, while a 20 mm wide crab readily consumes oysters 30 mm long. Maximum consumption rates of a 35 mm wide crab is two 25 mm long oysters in 6 hours. Oyster growers are now manually removing all *Cancer oregonensis* larger than 20 mm in carapace width. This control measure has drastically increased the survival of young oysters.

**ANALYSIS OF THE MICROSTRUCTURE AND FRAGILITY OF THE SHELLS OF PACIFIC GEODUCK *PANOPEA ABRUPTA* REARED IN ARTIFICIAL NURSERIES.** Donald E. Velasquez,\* School of Fisheries, University of Washington, Seattle, Washington 98195.

The state of Washington is currently involved in a program to enhance harvestable stocks of geoduck clams. With this goal in mind, three main problems have surfaced: consistent production of sufficient plantable seed clams, shell fragility in these clams, and very heavy predation mortality for the first year after planting.

This research is primarily directed toward the shell fragility evident in some groups of seed. The harvesting process can result in shell breakage up to 60% in some seed lots. Broken seed is currently believed to be slower to bury and more vulnerable to predation as a result. Shell quality of seed clams taken from two nurseries can be correlated to information obtained from scanning electron micrographs of valve sections. Seed reared at one Puget Sound nursery possess valves which are on average half as thick as those from another site in Puget Sound.

A secondary goal of this research is to provide basic information on geoduck shell structure. The three-layer construction and dissoconch spines of the juvenile geoduck shell will be discussed. Information from acid etched cross sections indicates microgrowth increments are difficult to discern in artificially-reared juvenile geoduck, possibly reflecting the stability of their nursery environment.

**DOES STARFISH REMOVAL INCREASE MUSSEL PRODUCTIVITY?** Sylvia Behrens Yamada,\* Bruce A. Menge, Eric L. Berlow, and Carol A. Blanchette, Zoology Department, Oregon State University, Cordley Hall 3029, Corvallis, OR 97331-2914.

The starfish *Pisaster ochraceus* is a major predator of the sea mussel, *Mytilus californianus*, on Pacific Northwest shores. *Mytilus californianus* forms extensive beds, up to 1 meter deep, in the mid- but not the low intertidal zone. Mussels that settle in the low intertidal grow better than in the mid-intertidal but rapidly fall prey to *Pisaster*.

The mussel industry in Oregon is based primarily on the harvest of wild populations of *Mytilus californianus* from mid-intertidal beds. Market size mussels in these beds grow only about 1 mm per month and thus are not regenerating as fast as they are being harvested.

We explored the possibility of opening up the productive low intertidal zone to mussel production by establishing 8 starfish removal and 8 control plots at two sites on the central Oregon coast. Ten cages of 50 mussels each, were transplanted to each of the 16 low intertidal plots. Once mussels attached themselves to the substrate with their byssal threads, the cages were removed. Survivorship of mussels was monitored and invading starfish were removed from the starfish removal plots every two weeks.

After two months the survival of mussels transplanted to the low intertidal was between 50 and 75% in starfish removal plots and between 0% and 75% in control plots. Very few mussels in any of the low intertidal plots survived one year because it was not possible to remove starfish during winter storms. Growth rates in these mussels ranged from 1 mm to 2 mm per month. These values fall in between the growth measured in mid-intertidal beds and on subtidal long lines.

Even though mussels grow better in the low than in the mid-intertidal zone, one would have to remove starfish at least every two weeks before permanent mussel beds could be maintained in the low intertidal zone. Since storms make the low intertidal inaccessible during most of the winter, it appears that starfish removal will not be a practical management tool for increasing mussel productivity.



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**COVER PHOTO:** American lobsters (*Homarus americanus*, H. Milne Edwards, 1837) ready for market. Photo by Jim Rollins.

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